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FACULTY of AGRICULTURE
UNIVERSITY of TOKYO

No. VIII (1957-1958)

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I. Agriculture

Abstract Nos. 1—23

1. Appearance of ear and flowering in the aseptic culture of excised stem tips in rice and wheat plants. Shin-ichiro KAWATA (Proc. Japan Academy. **33**, 474–476, 1957)

For several years, the author attempted to culture the excised stem tips of rice, *Oryza sativa* L. and wheat, *Triticum vulgare* Vill., and the flower buds were finally seen on the plants in vitro. One variety of rice, "Noorin No. 11" and two varieties of wheat, "Noorin No. 14" and "Saitama No. 27" were used as materials. Each of the stem tips was cut off 1-3 mm in length under the binocular microscope. Then each was transplanted in a tube, which contained the culture medium. The composition of mineral salts in the nutrient solution was essentially the same as that used by White and Bonner.

Seeds of rice plants were germinated under sterile conditions. On July 15, 1955, stem tips of the seedlings, composed of one growing point and two primordia of leaves were transferred aseptically to the culture tubes. On the 78th day from the first transplanting the ears appeared on the plantlets after the development of the 8th leaf. But the flowers failed to bear fruits and soon withered. Stem tips were cut from the young wheat plants when they were about 10 cm in height and transferred aseptically to the culture tubes on December 19th, 1955. On the 93rd day from the first transplanting the earing and blooming kere seen in the culture tubes. It may be expected that the mechanism of flower bud initiation in the cereals will be made clear to some extent with aseptic culture, controlled by artificial conditions, but physical and physiological, combined with photoperiodic treatment and vernalization.

2. Studies on the morphological characters and growth habits of safflower. Goro NISHIKAWA, Tozaburo MIKAMI and Shotaro KURODA (Proc. Crop Sci. Soc. Japan **26**, 51–52, 1957)

The principal results obtained were as follows. (1) There are varietal differences in the developing type of the plant, in the spine number on the bract and in the number of bract. (2) The flowers bloom out in order from the top of a main stem to its lower branches. (3) The flowers begin to bloom at 4 a.m., and at 6 a.m. the stigmas are already covered with pollens. (4) Generally, yellow flowered varieties contain larger amounts of oil than those in red flowered varieties. (5) The oil content in the seed, correlated decidedly to the hull percentage of it ($r=ca.\ 0.8$) (6) Fertilization is remarkably restricted by rain fall. The fact may be related to protandry. (7) For the growth of the plant, autumn sowing is better than spring one, and direct planting is better than transplanting.

3. Studies on CO_2 exchange in crop plants. Part 8. Relationship between the light intensity of normal and holizontal incidence and the photosynthetic rate of rice plants under natural condisions. Tomoshiro TAKEDA (Proc. Crop Sci. Soc. Japan **25**, 207–

209, 1957)

For studying photosynthetic rate of crop plants cultivated in the field, usually natural sunlight is used as the light source. However, the intensities of natural sunlight received by crop plants is not only subject to variations with the season of the year, the time of day, and the meteorological conditions, but also different representation of its intensities may be observed by the change of measuring method. Therefore, it may be expected that the relationship between the light intensity and the photosynthetic rate varies due to the different representations of light intensities.

Based on diurnal course of photosynthetic rate obtained by means of "infrared CO_2-analysing recorder", author made two kinds of light curves by arranging photosynthetic rate in accordance with the received light intensities of normal and horizontal incidences.

It can be represented better by arranging in accordance with the light intensity of horizontal incidence rather than the normal, with the light curve, which is of light unsaturating type. On the otherhand when the light curve is of light saturating type, it can be represented by arranging in accordance with either the latter or the former (regardless of isolated or community conditions).

4. **Analysis of grain production in rice plant. Part 1. Influence of nitrogen levels on leaf area, photosynthetic ability and respiration rate. Part 2. Influence of nitrogen levels on light receiving coefficient and dry matter production.** Tomoshiro TAKEDA and Atsuhiko KUMURA (Proc. Crop Sci. Soc. Japan **26**, 165–175, 1957)

Paddy plants of the variety Norin No. 29 were grown under various nitrogen conditions, and several aspects concerning the dry matter production were observed.

The relationship between dry matter productivity and the factors concerning CO_2 assimilation ability of rice plants, growing under community conditions and respiration rate were studied. From the experimental results the following conclusion was derived.

In general, the dry matter productivity of the rice community can be expressed as follows:

$$\Delta W = k \cdot L \cdot S \cdot p_0 \cdot \rho - R_W \qquad (k = \text{constant})$$

where, ΔW: denotes the dry matter produced per day, L: integrated light intensity, S: leaf area, p_0: mean photosynthetic activity per leaf area under saturating light intensity, ρ: light-receiving coefficient, and R_W: consumed dry matter by respiration.

5. **Studies on the production and behavior of carbohydrates in rice plant. Part 5. Influence of nitrogen on ripening.** Atsuhiko KUMURA (Proc. Crop Sci. Soc. Japan **25**, 214–218, 1957)

Under various nitrogen conditions rice plants, var. Norin-No. 29 were cultivated and influence of nitrogen on the ripening process was investigated.

Results obtained may be summarized as follows:

(1) Grain yield increased with the increase of nitrogen, supplied during ripening period, but too much nitrogen reduced the yield.

(2) Such effects of nitrogen were observed when the fertilizer was applied one week before heading time and 5 days after heading but such effects could hardly be observed when it was given 18 days after heading.

(3) The plants which were deficient in nitrogen were inhibited rather in the later process of ripening than the earlier, but reverse result was observed in the plants which had an abundant supply of nitrogen.

(4) The inhibition of ripening under nitrogen deficient condition was due to the decrease in photosynthetic activity of leaves but under excessive nitrogenic condition the inhibition of ripening was due to the inhibition of translocation of photosynthesized matter to panicle.

(5) The lowest nitrogen level, an average of about 1.0-1.4%, from 5 to 35 days after heading, should be maintained in the leaf blade in order to obtain the best ripening.

6. Studies on embryo culture in plants. Part 1. On the action of auxin and embryo factor on embryonic growth. Tetsuo NAKAJIMA (Japanese Journ. Breed. 7, 161-168, 1958)

It is necessary to make clear the action of embryo factor which is closely related to growth of the very young embryo in plants, for establishing the technique of vitro culture of the plant embryo. In this report, the author has studied action of auxin and embryo factor on embryonic growth.

The growth of embryo of cucumber in a medium containing malt extract or *Cucurbita* endosperm extract, showed the existance of embryo factor in malt extract and also *Cucurbita* endosperm extract. By means of avena curvature method, it was shown that embryo and endorsperm of *Cucurbita maxima* contained the free auxin and bound auxin (alkali released) at all stages of seed development. And also the above mentioned malt extract and endosperm extract of *Cucurbita* contained both the free and bound auxin. Therefore, the author thinks that the auxin plays a very important role in the growth of embryo.

Then the growth of embryo of cucumber (late heat stage-early torpedo stage) in a medium containing IAA and also in a medium containing embryo factor has been compared from morphological and histological point of view. In morphological observation, the former (only high concentration; IAA 2 ppm.) was similar to the later. In histological observation, only cell elongation was recognized in using IAA, but in using embryo factor, both the cell elongation and cell division were recognized. And then, the existance of starch grain in the cells of cultured embryo showed that the action of embryo factor is related to the action of auxin. So the author thinks the embryo factor contains auxin and other factors which accelerate the cell division in plant embryo.

The author cultured the cucumber embryo in a medium containing kinetin or 1,3-diphenylurea, which are substances promoting cell division. In adding kinetin, no conspicuous action was recognized, but in adding 1,3-diphenylurea, conspicuous effects were recognized viz. the cultured embryo in the medium containing 1,3-diphenylurea showed seedling growth, and that in the medium containing 1,3-diphenylurea with IAA showed the inhibition of seedling growth and slightly embryonic growth. And when casein hydrolysate was added, the effects of 1,3-diphenylurea were more conspicuous, however, in the case with IAA, sometimes callus-like mass was recognized. So, the author thinks that the action of auxin is necessary to embryonic growth, even with the existance of the substance promoting cell division.

In conclusion, the author thinks that the action of embryo factor is an action synthesized by auxin, a substance or substances promoting cell division and nutrient source which is similar to casein hydrolysate.

7. Studies on the mulberry-blight. Part 1. On the physiological characteristics in relation to the mulberry-blight. Yoshiro ONOZAWA (Japanese Journ. Breed. 7, 186–190, 1958)

Some varietal differences of the chemical constituent in the barks of trees in relation to the resistance to the mulberry-blight were investigated. The results obtained are summarized as follows:

(1) Quantity of the total carbohydrate in the bark was not much changed regardless of variety, locality and season (at both the times after falling of leaves and thawing).

(2) Tendencies in decrease of total sugar content were found in all materials, especially in susceptible varietes, the remarkable decrease in the total sugar occurred accompanied by an increase in insoluble carbohydrates during winter.

(3) It was obviously noticed that the insoluble carbohydrate content/total sugar content ratio in the bark at the time after falling of leaves was higher in resistant varieties than in susceptible ones. In other words, the barks of resistant varieties are more advanced in degrees of hardening than those of susceptible at this time. Similar analytical result was found between nitrogen fractions.

(4) No correlation was found between water content and the blight resistance.

(5) From the results mentioned above, it was suggested that the degree of debility of mulberry-tree under snow was closely related to the physiological characteristics of varieties before snow cover.

Part 2. A segregating pattern of physiological characteristics in F_1 hybrids between resistant varieties and susceptible ones. Yoshiro ONOZAWA (Japanese Journ. Breed. 7, 231–234, 1958)

Previously, author reported that the ratios of insoluble carbohydrate content to total sugar content in living bark was higher in resistant varieties than susceptible ones at the time after the fall of leaves. This result seemed to be of interest for the study of the physiological mechanisms of blight resistance and breeding of the resistant strains. In present studies, segregating pattern of this characteristic of F_1 hybrids in 8 crosses between resistant varieties and susceptible ones were investigated. These results obtained were summarized as follows:

(1) Parental varieties of all crosses (except one cross) showed the parallel results with previous finding.

(2) Hybrid individuals in each cross contained varieties of carbohydrate composition. And those indicated continuous distribution, having peaks in mid parents.

(3) Hybrid individuals in two crosses between cultivated and wild, the correlation between the degrees of resistance from field test and physiological characteristics has been shown as same as observed between varieties in on cross, but in not others. Thus, to secure a clear conclusion in this problem, further investigations are necessary with in many crosses and a large number of hybrids.

8. X-ray experiments on dormant seeds of six-rowed barley. Hikoyuki YAMAGUCHI (Japanese Journ. Breed. 7, 175–178, 1958)

In this experiment the variety chosen was "Sekitori Sai No. 1", a six-rowed barley, now widely cultivated in Japan. Dormant seeds which had been stored under laboratory conditions since the previous harvest were used for irradiation. The seeds were placed in open petri dishes at about 9 cm. from "target" of a 60 kV x-ray apparatus, run at 4 ma, where the dose rate was 235 r. per minute. After doses of 435r to 25,000r were given, the seeds were sown in the field together with non-irradiated seeds from the same sources. The seedling growth determined by measurements of the total height of the 18 day plant after sowing was inhibited with the increasing dose of x-ray. As regards the shape of the growth/dosage curve, the x-rays gave a sigmoid-curve. The average spike fertility, as evidenced by seed-setting of the irradiated generation, decreased due to x-irradiation. Regression analysis indicated that the average spike fertility was a linear function of x-ray dosage. About three spikes were picked up from each plant, and the seeds were sown for seedling mutation studies. The observed mutation resulted from chlorophyll deficiencies, and a linear relation between dose and frequency of mutation was obtained. While at low doses the frequency of mutations per spike progeny and per dose unit was almost constant, at higher dose there was a drop in the amount of effect produced per dose unit. Also, the phenotypic effects differed in response to varying doses, *i.e.*, the mutation frequency of *viridis* increased linearly with dose, while that of *albina* showed a similar linear response to lower doses but somewhat decrement in the frequency of mutation at high doses. Most of the mutants were produced in the same relative frequencies as reported by earlier workers. In this experiment, it was calculated that dose of x-ray necessary to produce 2.5 per cent chlorophyll deficient mutations per spike progeny was 6,000r.

9. Comparison of the effects of mustards and x-rays on the mutation of rice. Hikoyuki YAMAGUCHI (Japanese Journ. Breed. 7, 179–185, 1958)

Dormant seeds of a rice variety, "Norin No. 29", were treated with solutions of nitrogen mustard: methyl-bis-(di-chloroethy)-amine hydrochloride, or of its N-oxide derivative, or with X-radiation. Nitrogen mustard presented a more toxic effect on germinating ability than in the case of X-ray or N-oxide treatment. While X-ray and/or N-oxide gave sigmoidal curves, as regards to the relation between germination and dosage, nitrogen mustard showed an exponential one. A *chi*-square-analysis performed after a correction of discontinuity gave the indication on mutagenic action of nitrogen mustard. In comparison with ionizing radiation (X-ray or fast neutron) and N-oxide, nitrogen mustard showed a significant difference in its ability to induce the mutations of *striata*-type. When dormant seeds of rice were exposed to N-oxide of nitrogen mustard, however, it showed the same tendency in the sterility of the treated generation, the production of seedling mutants and the relative frequency of chlorophyll mutations, as found in X-rayed material. For seeds, N-oxide must be considered as a less favourable mutagen than X-radiation, for the former's toxic action.

10. Comparative mutagenic action of x-rays and cyclotron neutrons on dormant seeds of rice. Hikoyuki YAMAGUCHI (Radioisotopes 7, 53–56, 1958)

In this experiment, the effects of x-rays and fast neutrons on rice seeds were studied and the results compared. Dormant seeds of paddy rice "Norin No. 29" were irradiated. The x-rays were shot at 60 kV without filtration. The neutrons were produced from the bombardment of a Be target with ~4 Mev deuterons, accelerated in a 66 cm cyclotron. During the treatment, the seeds were put in a glass tube, and sulphur foils along with the material were exposed to the neutron beam and their induced beta-radiation subsequently estimated with a Geiger counter. By the resulting beta-ray counts, the fast neutron flux was estimated. In higher neutron irradiation ($3.5\times10^{10} n_f/\mathrm{cm}^2$), germinability determined thirty days after the time of sowing, was as high as that of no-treatment. As regards growth inhibition, there is qualitatively a difference between the two radiation. While neutron has a uniform action on the seeds, there seems to be much difference between the sensitivities of each seeds of x-ray. The reduction of spike fertility was directly proportional to the dose of both radiation. In the next generation the chlorophyll deficient mutants appeared in the seedling stage. And the frequencies of seedling mutation were a linear function of the dose of x-rays and neutrons, and, however, direct proportionality of frequency of seedling mutation to the dose of x-rays was depressed somewhat at high doses. It seemed that as many mutants were induced with x-rays as the fast neutrons, comparing in the same spike fertility. In terms of the production of 2.5 per cent seedling mutation per spike progeny, it was calculated that approximately $3.5\times10^{10} n_f/\mathrm{cm}^2$ were equivalent to 6,000r from x-ray.

11. The effects of fractional x-ray dosage on sterility and mutation in dormant seeds of rice and barley. Hikoyuki YAMAGUCHI (Japanese Journ. Breed. 7, 213–220, 1958)

In order to clarify the effect of fractional dosage on sterility in the treated generation, dormant seeds of a variety "Norin No. 29" of rice and of a variety "Sekitori Sai No. 1" of barley were treated with x-ray. The irradiation was given in two fractions of 10,000r at 1, 2 or 4 days interval and a single dose of 20,000r in both species. The plants treated with the fractional dosage produced more sterility than those of the single dose in the first generation of rice as well as of barley, and the increment of the rest period elapsing between first irradiation and the second induced more sterile spikes. This result obtained with dormant seeds is probably due to the storage of seed after the cessation of x-raying and also to the sensibility of the seeds affected by the first treatment.

In barley, the frequency of chlorophyll deficient mutation and the survivals at maturity showed no change among fractional dosages-and a single dose-treatment. Thus, it is thought that mutation in barley, too, is induced by 1-hit event, as has been interpreted generally. However, the fractional dosage-irradiation gave a different relative frequency of chlorophyll mutants as compared with a single dose-treatment. The former induced more *viridis* type than *albina* mutant, at rest period of two or four days. On the other hand, *viridis* type was present frequently in those first generation spikes which showed some infertility (more than 20% sterility), while there was no difference in the frequency of chlorophyll mutation between the fertile and the infertile. Hence, it is assumed that a difference of relative frequency of mutants is due to the sterility and *viridis* type may be accompanied with the sterility.

12. Intervarietal variation of some characters among varieties of upland rice cultivated in Japan. Hikoyuki YAMAGUCHI and Sadao KIMURA (Japanese Journ. Breed. 7, 241–246, 1958)

To study the phylogenic differentiation of upland rice cultivated in Japan, more than 130 varieties were picked out from numerous varieties collected from various districts by Agricultural Experimental Stations, belonging to the Department of Agriculture and Foresty, and investigated with respect to varietal variation of some characters. Most of the varieties of upland rice of Japan showed positive phenol reaction, while lowland rice showed negative. Some characters, *i.e.*, the degree of destruction of endosperm by KOH solution, the length of mesocotyle grown in a dark place and the length-width ratio of unhulled grain showed continuous varietal variation. It seemed that upland rice of Japan was composed of varieties of different nature as compared with lowland rice of Japan. Grain type of upland rice of Japan were a-, ab-, b-, and bc-types (MATSUO 1952). Varieties possessing grain type b were found distributed mainly in mountainous parts of Formosa. Varieties possessing bc type were found among native varieties cultivated in the mountainous parts of the Philippine Islands. Minus intervarietal correlation was very close between the length-width ratio of unhulled rice and the length of mesocotyle, and also the length-width ratio of unhulled grain and the degree of destruction of endosperm by KOH solution. With regard to each of the characters there appeared continuous varietal variation and its range was divided into two parts, designated as + and —, and thus the characteristics of a variety was represented by a combination of three plus or minus signs. The frequency of varieties possessing each kind of those combinations was investigated, and due to OKA's classification of Insular variety-group (OKA 1953) the varieties of upland rice of Japan were to be divided into two minor groups, that is, "Tropical Insular" and "Temperate Insular". Within the upland rice of Japan, temperate insular group was found to be preponderant. Varieties estimated as tropical insular group are distributed mainly over the southern region of Japan.

13. Studies on a mosaic disease of squash in Japan. Part 2. Physical properties and identification of its causal virus, with special reference to the relationship to the virus from mosaic plants of white gourd, oriental pickling melon and watermelon. Yasuo KOMURA (Ann. Phytopath. Soc. Japan **22** [4–5], 220–224, 1957)

The virus isolated from mosaic plants of squash, the symptoms and host range of which were described in the previous paper, withstood 50°C for 10 min., 1/500 dilution and 5-days' aging in vitro. It was inactivated in extracted sap by 55°C for 10 min., by 1/500 to 1/1000 dilutions or by 5- to 10-days' aging at room temperature. It failed to protect cucumber and squash plants from infection by cucumber mosaic virus, showing that the virus is not allied to the cucumber mosaic virus.

The virus was compared with the description of those viruses that have been reported to be infective principally on cucurbits, and was identified to be a strain of the melon mosaic virus designated by Lindberg et al. in 1956. It may be closely related to the bottle gourd mosaic virus described by Vasudeva and Lal in India, or to the cantaloupe mosaic virus reported by Aycock, or to the watermolon mosaic virus described by Anderson in U.S.A.

From mosaic plants of white gourd, oriental pickling melon and watermelon, a virus was recovered, being very similar with the present virus in pathogenicity.

14. Studies on the mechanism of action of insecticides. Part 16. Nervous activity as a factor of development of dieldrin symptoms in the cockroach. Teruo YAMASAKI and Toshio NARAHASHI (Botyu-Kagaku 23, 47–54, 1958)

The effects of dieldrin on nervous activity of the cockroach were studied in order to clarify the mechanism of development of dieldrin poisoning symptoms.

The course of poisoning symptoms of dieldrin was as follows: latent period, period of ataxia, convulsive period, paralytic period and death.

Spontaneous activity in the abdominal nerve cord increased markedly and synaptic after-discharges in the sixth abdominal ganglion prolonged remarkably in the cockroach showing ataxia. The synaptic after-discharges were maintained at the prolonged level when the poisoning symptoms advanced to the initial stage of paralysis, while the enhanced spontaneous activity in the abdominal nerve cord progressively decreased during that period.

The increase in spontaneous activity in the abdominal nerve cord and the prolongation of synaptic after-discharges were also found when the dieldrin suspended Ringer's solution was applied directly to the nerve.

The sensory discharges in the crural nerve showed no sign of changes in the cockroaches developing ataxia or convulsion, or following injection of dieldrin suspension in the leg.

The mechanism of development of dieldrin poisoning symptoms was discussed compared with those of DDT and BHC which had been reported previously. The poisoning symptoms of dieldrin are different from those of both DDT and BHC. Prolonged synaptic after-discharges and increased spontaneous activity make it difficult for the cockroach to coordinate its movement, causing ataxia. Further progress of such changes in nervous activity makes it quite impossible for the cockroach to coordinate its movement, causing knockdown and convulsion. Paralysis of the cockroach is caused by the progressive decline in nervous activity with the advance of time.

The effects of dieldrin upon nerve were less striking and more moderate than those of BHC, which seems to be partly responsible for slow insecticidal action of dieldrin.

Part 17. Resistance of house flies to insecticides and the susceptibility of nerve to insecticides. Teruo YAMASAKI and Toshio NARAHASHI (Botyu-Kagaku 23, 146–157, 1958)

A number of detailed studies have been made on the mechanism involved in the resistance of insects to insecticides. There is a general agreement that DDT-resistant strains of house flies detoxify DDT more effectively than DDT-susceptible strains. Furthermore, there is evidence suggesting some additional defense mechanisms. In fact low sensitivity of nerve to DDT has been demonstrated in some DDT-resistant strains. However, few data are available for Japanese resistant strains. The present investigation was undertaken to gain information concerning the mechanism of DDT, BHC and dieldrin resistance of the Hikone strain of house flies.

The toxicities of *p,p'*-DDT, γ-BHC and dieldrin were estimated by topical appli-

cation using the Takatsuki and the Hikone strains of house flies. DDT was extremely ineffective against both strains, the values of LD50 in both strains being much more than 5000 γ/g at 30°. Both BHC and dieldrin were less effective to the Hikone strain than to the Takatsuki strain. The values of LD50 for BHC in the Takatsuki and the Hikone strains were 10.0 γ/g and 51.1 γ/g respectively at 30°, and those of dieldrin were 2.38 γ/g and 96.4 γ/g respectively at 30°.

The methods of recording the discharges from the thoracic ganglion were studied. It was found that the recording of the nerve action potentials from the femur is the most suitable method for estimating the activity of he exposed thoracic ganglion under the influence of drugs applied directly to it.

With this method the effects of *p,p'*DDT, γ-BHC and dieldrin on the activity of the thoracic ganglion were studied. Any of these insecticides caused the ganglion to initiate the intermittent bursts of discharges, the latent period for these effects being dependent on both insecticides and strains.

The latent period for the initiation of the bursts following treatment with dieldrin was longer than that following treatment with about the same concentration of BHC in each strain of house flies.

The nerve of the Hikone strain is only slightly less sensitive to DDT than that of the Takatsuki strain. The nerve susceptibilities of the Hikone strain to both BHC and dieldrin as determined by the latent period for the initiation of the bursts were lower than those of the Takatsuki strain. These differences in the nerve susceptibility were considered to play a part in the resistance of the Hikone strain to BHC and dieldrin.

15. Zwei *Heminothrus*-Arten aus Japan (Acarina: Oribatei). Jun-ichi AOKI (Ann. Zool. Jap. **31**, 121–125, 1958)

Two species of mite, i.e. *Heminothrus paolianus* (Berlese), var. *longisetosus* WILIMANN and H. YAMASAKII n. sp. (Family Camisiidae) were recorded and described The former is only known in Europe, and recorded for the first time from our country. The latter is a new species, collected from Utsukushigahara, Nikko and Tanzawa. A key to the species of this genus in the world, is also given.

16. Einige Phthiracariden aus Utsukushigahara, Mitteljapan (Acarina: Oribatei). Jun-ichi AOKI (Ann. Zool. Jap. **31**, 171–175, 1958)

Four species of the family Phthiracaridae were found in the collection of soil-mites from Utsukushigahara plateau. Two of them have been recorded recently in Japan and other two are new species. They are *Phthiracarus japonicus* n. sp., Pseudotritia ardua (C. L. KOCH), *Stegenacarus senex* n. sp., and *Stegenacarus striculus* (C. L. KOCH).

17. Mechanism of resistance to virus diseases in the silkworm, *Bombyx mori*. Part 2. On the relation between the nuclear polyhedrosis and the cytoplasmic polyhedrosis. Hisao ARUGA (Journ. Seric. Sci. Japan **26**, 279–283, 1957)

Studies on the nuclear polyhedrosis (jaundice disease) and the cytoplasmic polyhedrosis (mid-gut polyhedrosis) in the silkworm have been carried out by several races and their hybrids. It may be plausibly thought that the virus of the cytoplasimc

polyhedrosis is different from that of the nuclear type, confirmation being made by feeding-experiments with polyhedra-plastered mulberry leaves, the position difference in their formation, and the stainability of the polyhedral bodies.

In numerous larvae, cytoplasmic polyhedrosis could be induced by low-temperature (5°C, 24 hours) treatment of the larvae just after ecdysis, particularly in the 5th larval stage as in the case of the nuclear polyhedrosis.

The mid-gut polyhedral virus may be imagined to be due to a mutation of the nuclear type, whence the former has acquired the characteristic of multiplying more readily in the cytoplasm of the cylindrical cells of the mid-gut than the mid-gut polyhedral virus reported by ISHIMORI (1934).

Part 3. Histological studies on the polyhedrosis in the silkworm. Yoshimitsu IWASHITA and Hisao ARUGA (Journ. Seric. Sci. Japan **26**, 323–328, 1957).

Histological and histochemical studies on the polyhedral diseases, particularly on the relation between the process of the formation of polyhedra and nucleic acid, have been carried out with various staining techniques. The results obtained are as follows:

1. Polyhedral bodies are formed in the cell nuclei of oenocyte, muscle, basement membrane and epithelial cells of the mid-gut of nuclear type oplyhedral diseased larva.
2. The process of the polyhedral formation in the cytoplasm of cylindrical cells of the mid-gut was investigated.
3. In general the cytoplasmic type polyhedra are formed in the cytoplasm of cylindrical cells, but in a few cases they are formed in the goblet cells, too.
4. Regenerative cells of the mid-gut located in *nidi* at the base of the digestive cells are not susceptible to virus infection, but in a few of them polyhedral may be formed when they develop, accompanying the degeneration and destruction of the epithelial cells.
5. Cross infection of both the nuclear and the cytoplasimc types was observed in the mid-gut epithelium of the first instar larva.
6. Nuclear type polyhedra showed a positive Feulgen reaction, indicating the presence of desoxyribose nucleic acid (DNA), but cytoplasmic ones showed a negative reaction.
7. The stainability difference between the nuclear and cytoplasmic polyhedra was investigated by applying a new method of staining with orange G-aniline blue.

Part 4. On the relation between the polyhedral diseases and environmental factors. Hisao ARUGA (Journ. Seric. Sci. Japan **27**, 5–9, 1958)

A comparative study on the percentage of appearance of cytoplasmic and nuclear polyhedrosis in the silkworm reared at the University of Tokyo and the Shinshu University (Ueda, Nagano Prefecture), basing upon the probable difference in the quality of mulberry leaves grown at the two respective places, using several resistant and susceptible strains and their hybrids, was made. The results obtained are summarized as follows:

1. In the experiments conducted during the spring rearing, in which the low-temperature treatment (5°C, 24 hours) was given to the 5th instar soon after ecdysis, cytoplasmic (mid-gut) polyhedral diseased larvae appeared less at Ueda than at Tokyo.

2. In the case of summer rearing in which low-temperature treatment was not applied, a result similar to the above was obtained, i.e. the number of larvae affected by mid-gut polyhedral disease was less at Ueda than at Tokyo.

3. The percentage of mid-gut polyhedral diseased larvae induced by the low-temperature treatment in the summer rearing was higher at Tokyo than at Ueda.

4. The larvae reared first at Ueda for the 1st and 2nd instar and then at Tokyo from 3rd to 5th instar, showed an intermediate value of mid-gut polyhedrosis between those reared at Ueda and at Tokyo, respectively from the 1st up to the 5th instar.

From these results, it may be plausibly thought that some environmental factors, in this case the quality of mulberry leaves, markedly influence the appearance and induction of the cytoplasmic polyhedrosis in the silkworm.

Part 5. On the induction of the polyhedral virus by nitrogen mustard. Hisao ARUGA (Journ. Seric. Sci. Japan 27, 10–13, 1958)

Studies on the induction of nuclear and cytoplasmic (mid-gut) polyhedral viruses utilizing nitrogen mustard, X-rays, ultraviolet rays have been carried out on the silkworm with a few susceptible and resistant races, hybrids and *od* oily mutant.

As numerous diseased larvae in the 5th instar appeared, typically displaying the formation of polyhedral bodies in the cytoplasm of cylindrical cells in mid-gut by the nitrogen mustard treatment (feeding and injection) in the 3rd, 4th and 5th instars, it may be concluded that the cytoplasmic polyhedral virus can be induced by the nitrogen mustard treatment. The nuclear polyhedral bodies observed in a hybrid treated by nitrogen mustard showed tetragonal configuration, different from normal polyhedra, which are hexagonal.

The phenomenon of the induction of both the nuclear and cytoplasmic polyhedral viruses was not recognized in the experiments of X-ray and ultraviolet ray treatments.

18. Studies on the isoallelic genes in the silkworm, *Bombyx mori* L. Part 1. Mutant isoalleles at the $+w_1$ gene locus in the silkworm. Narumi YOSHITAKE (Jap. Journ. Genet. 33, 81–86, 1958)

The egges laid by white-1 strain (female) mating with normal strain (male) are pale colored. This pale coloration is due to the fact that a part of kynurenine which exists in white-1 strain egg is changed into 3-hydroxykynurenine and then into the pigment by the function of $+w_1$ gene of the male strain. However, there are cases in which white-1 strain is colored scarcely or deeply, according to the different strain of the male used. An analysis of genetical causes of such different pigmentations proved that the genes which control the pigmentation are closely linked with white-1 gene. Moreover, after determining their positional relations with w_2 and w_3 located on 10th chromosome, this kind of genes were ascertained to be multiple alleles or pseudoalleles of w_1 gene. From these results it has become evident that $+w_1$ gene consists of a number of isoallelic genes having different expression in strength and that $+w_1$ gene differs in a few strains.

Part 2. Nutant isoalleles at the $+w_3$ gene locus in the silkworm. Narumi YOSHITAKE (Jap. Journ. Genet. 33, 102–106, 1958)

Among the so-called normal eggs of the silkworm there are some with deep colored and some with pale colored serosal cells. The causes producing such a variation are attributed to difference in the amount of 3-hydroxykynurenine and the differential enzymatic action. In this connection, by making an analysis of the gene controlling this enzyme activity, the author has found the $+w_3$ gene to be the most important. On the locus of $+w_3$ gene exist several kinds of isoallelic genes and their activities differ more or less quantitatively. One of the causes which change the color tone of serosal cells in normal strains is considered to be this difference of the $+w_3$ gene.

19. Physiological and genetical studies on the integument in Lepidoptera. Part 4 Studies on factors controlling the uric acid content in the integument of normal and translucent silkworms, *Bombyx mori* L. Hitoshi WATANABE (Journ. Seric. Sci. **27**, 45–54, 1958)

1. Uric acid content measurements were made in the larval integument of silkworms in the 5th instar, using several normals and translucent mutants including individuals segregated in F_1 or F_2 generations of their hybrids.

2. The content of uric acid in the integument of distinct translucent (*od*), sexl-niked mutant, was as much as 31% of normal, and in Chinese translucent (*oc*) 62% of normal. Also, large intra-strain differences on the content were encountered in normal or in two mutant silkworms. Variability of the uric acid content in the larval integument, observed among strains of the same mutant or normal, was larger in *od* mutant than either in *oc* mutant or in normal; while it was the smallest in normal of the three.

3. The uric acid content in the integument of the 5th instar larvae, reared at low temperature (18°C) during the young larval stages (1st-3rd instars), showed decreased value as compared to those reared at high temperature (25°C). In this case, the decrease of the content in *od* mutant was much greater than normal.

4. Uric acid of blood in larvae, newly entered in the 5th instar, was much increased in content by the high temeprature shock treatment (temperatuer 38°C, relative humidity 80%, treatment for 12-24 hours) performed during the 4th moulting stage, but the quantity in the integument was not so much affected. Similar result was shown on the uric acid content in the integument, morphologically injured by ultraviolet light treatment: the content was almost as stable as before the treatment.

5. On the uric acid content in the larval integument of F_1 hybrid, maternal effect was observable, showing much larger quantity in F_1 hybrid (large contett strain female × small content male) than in the reciprocal cross.

20. Magnesium deficiency in vegetable crops. A survey in Fijimi-mura and Showa-mura, Yamanashi Prefecture. Tadayoshi SUGIYAMA, Kazuhiko TAKAHASHI and Yuji TOKUNAGA (Journ. Hort. Assoc. Japan **25**, 77–84, 1956)

In recent years, a kind of chlorosis has been widespread in many kinds of vegetable crops grown on sandy and gravelly soils near Kofu City, Yamanashi Prefecture. The authors surveyed those areas and attempted to elucidate its cause by chemical analyses of the plants and soil.

Tomatoes, egg-plants, peppers, cucumbers, melons, snap beans, chinese cabbages,

leafy brassicas, etc. suffered from this defect. The general symptoms were chlorosis of interveinal portions of the lower leaves, subsequent necrosis of the chlorotic areas, and in some crops premature defoliation. These symptoms were similar to the ones of magnesium deficiency of those crops artifically induced in sand culture in our laboratory and reported by other investigators.

Chemical analyses of the leaves of tomatoes and egg-plants revealed that magnesium contents were generally low in the affected leaves: less than 0.3% Mg in tomatoes and less than 0.25% in egg-plants.

The soils in those areas were sandy or gravelly, and very low in clay and organic matter contents. Soil pH ranged from 5.09 to 7.95; in most of the soils tested it was nearly neutral.

Exchangable capacities were generally low; most of them were 5-7 m.e. per 100 g, but some of them were below 5 m.e. Exchangable magnesium contents of the deficient soils were less than 0.5 m.e. per 100 g soil. No cases of magnesium deficiency due to high levels of potassium were found. From those results, it was clear that magnesium deficiency of vegetable crops in those areas was due to the low content of available magnesium in the soil.

It was found that there were differences in susceptibility to magnesium deficiency among vegetable crops; for example tomatoes and egg-plants were more susceptible than cucumbers. Furthermore, varietal differences were found in resistance to the disorder in some crops.

21. On physical and chemical properties of the composts for raising vegetable seedlings in hot beds. Tadayoshi SUGIYAMA and Kazuhiko TAKAHASHI (Jour. Hort. Assoc. Japan **26**, 223–229, 1957)

The composts for raising vegetable seedlings in hot beds are generally prepared by mixing soil, manure, and fertilizers in proper portions, and leaving them to mature in pile for more than a half year before using. Since no standard formula is available for preparing the composts in relation to the portions of soil, manure and the amounts of fertilizers to be added, the authors intended to know the physical and chemical properties of excellent composts. Twentyfive samples were collected from the gardens raising good vegetable seedlings, and were analysed. The results of the analysis showed the following facts.

One of the most important factors in relation to the texture and other properties of the composts was the organic matter content (loss of ignition). The higher the organic matter content, the lower the specific gravity and the apparent specific gravity, and the higher the porosity, moisture equivalent, total nitrogen, and exchangable capacity.

The composts for raising cucumber seedlings contained higher levels of organic matter (more than 20 percent in loss of ignition), while the ones for tomatoes contained rather less (12.1-18.7 percent). The organic matter contents in the composts for egg-plants were variable, ranging from very low to high (6.5-22.8 percent).

Hydrogen-ion concentrations of the composts were in the range of pH 5.2-8.0, but most of them were in pH 7±1, or nearly neutral in their reaction.

Available phosphorus contents were determined by the Truog's method and

Bray's method. The amounts of the available phosphorus found in the samples were in the range of 41 to 1,110 ppm by the Truog's method, and 198-917 ppm by the Bray's method.

All of the samples except one contained more than 1.0 m.e. of exchangable potassium, and three of them contained more than 10 m.e. of it.

It was not clear how much of P and K levels were sufficient for the excellent growth of vegetable seedlings, but the amounts in some of the samples, at least, were so high, that they seemed to contain surplus amounts of fertilizers.

It was found that fairly large amounts of nitrates and ammonium nitrogen had accumulated in most of the composts tested.

22. Influence of the forms of nitrogen supplied on the growth of vegetable crops. Part 2. Effects of varying concentrations of nitrogen in the different forms on the growth of turnip. Masatoshi IWATA (Journ. Hort. Assoc. Japan 27, 21–31, 1958)

Experiments were carried out to clarify the influences of the forms of nitrogen supplied with varying concentrations on the growth, yields and chemical compositions of turnip in sand culture under glass. In the first experiment, mixed nitrates (KNO_3:$Ca(NO_3)_2$:$Mg(NO_3)_2$=5:5:4), $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$ and $(NH_2)_2CO$ were used as nitrogen sources and nitrogen concentrations were 5, 10, 20, 40, 80 and 120 m.e. for each nitrogen source, respectively. In the second experiment, nitrogen sources were $NaNO_3$, NH_4NO_3 and $(NH_4)_2SO_4$, and nitrogen concentrations were 1, 3, 5, 10, 20, 40, 60, 80 and 120 m.e. All known nutrients were provided for each treatment. Results obtained are as follows.

(1) Among mixed nitrates, $NaNO_3$ and NH_4NO_3 treatments, there was no great difference of their top growth up to 40 m.e. (560 ppm) nitrogen level. But beyond 40 m.e., the decreasing of growth was most remarkable in the NH_4NO_3, and least in the mixed nitrates. The growth of plants supplied with $(NH_2)_2CO$, especially with $(NH_4)_2SO_4$ were markedly inferior to the above 3 nitrogen forms at all concentrations. And at higher concentrations of $(NH_4)_2SO_4$ and of NH_4NO_3, the decreasing of fleshy root growth was more remarkable than other nitrogen forms, and their fibrous roots also were injured.

(2) Total nitrogen contents of both leaves and roots increased with increment of nitrogen supplied, irrespective of nitrogen forms. And in the higher nitrogen concentrations (beyond 40 m.e.), increment of nitrogen content was remarkable for $(NH_4)_2SO_4$ and NH_4NO_3 treatments, but gradual for mixed nitrates and $NaNO_3$. $(NH_2)_2CO$ plants at lower concentrations contained less nitrogen than other nitrogen forms, but higher concentrations absorbed fairly much nitrogen. And generally $NaNO_3$ plants accumulated much of nitrate nitrogen, but $(NH_4)_2SO_4$ plants contained only trace, and NH_4NO_3 was intermidiate. As to ammonium nitrogen, the relation of $(NH_4)_2SO_4 > NH_4NO_3 > NaNO_3$ was found, but accumulation of ammonium nitrogen was much less compared with nitrate nitrogen.

(3) In the NH_4NO_3, especially in the $(NH_4)_2SO_4$ treatment, phosphorus contents of leaves was higher and increased with increment of ammoniacal nitrogen supplied. As to potassium, plants supplied with mixed nitrates or $(NH_2)_2CO$ contained much. Plants supplied with $NaNO_3$, NH_4NO_3 or $(NH_4)SO_4$, had less potassium contents than the above 2 nitrogen forms, and potassium decreased against in-

creased $Na^{\cdot}$ or $NH^{\cdot}_4$ supply. In comparison of calcium and magnesium contents among $NaNO_3$, NH_4NO_3 and $(NH_4)_2SO_4$ treatments, the tendency of $NaNO_3 > NH_4NO_3 > (NH_4)_2SO_4$ was found, so antagonism between $NH^{\cdot}_4$ and $Ca^{\cdot\cdot}$ or $Mg^{\cdot\cdot}$ was clear.

(4) Ascorbic acid contents in the leaves of $NaNO_3$ plants was high, and that of NH_4NO_3 plants was slightly less compared with $NaNO_3$ plants, at higher nitrogen concentrations. In the $(NH_4)_2SO_4$, ascorbic acid contents was much less and this tendency became more remarkable with the increasing of ammoniacal nitrogen supplied.

23. Studies on the Planning Standard of Landscape Design in Housing Estates. Mitsuo YOKOYAMA, Akira HONMA and Hisao FUKUTOMI (City Planning Review, 4, 2-11, 1957)

Recent examples of the housing estates of the medium height flats show that layout of each building is generally planned under the minimum condition to enjoy more than 4 hours sunlight at the winter solstice day. Of course, this kind of layout is not in accordance with the present standard of town planning. The authors, first analised the actual examples of the housing estates, and examined practical methods for realizing better residential surroundings from the view points of communal facilities, arrangements of trees, and landscaping of the estates and proposed a possible trial design.

The desire for outdoor open space among the flat dwellers varies according to the tastes of each family and length of leisure time and all this is under various influences of different floor. However, the most desirable way to realize better landscaping is to introduce communal gardens instead of common gardens through the positive corporation of the residents of the flats. The design of these enclosed communal gardens must appeal the residents and, at the same time, the superintendents of these gardens must discharge their duties to lead the residents in accordance with the whole picture of landscape of the estates. For this purpose proper guidance and assistance must be given to these superintendents.

II. Agricultural Chemistry

Abstract Nos. 24—58

24. On the artificial humic acids prepared from coal and microorganism pigments. Kyoichi KUMADA and Saburo HOTTA (Soil and Plant Food 3, 148-151, 1958)

In order to obtain artificial humic acids comparable to soil humic acids, the writers prepared humic acid-like substances from coals of various ranks and from the pigments poduced by *S. viridochrom* and *Botrytis cinerea*.

Coal humic acids, especially the ones obtained by potassium chlorate-nitric acid pretreatment were very similar in the absorption spectrum and stability to soil humic acids. On the other hand, microorganism pigment humic acids were considered not to be so analogous to soil humic acids as is the case of coal humic acids,

but might be their forerunners.

25. The infra-red spectra of humic acids. Kyoichi KUMADA and Kazuyuki AIZAWA (Soil and Plant Food 3, 152–159, 1958)

The infra-red spectra of various soil and artificial humic acids were determined using KBr-disk method. The results are summarized as follows:

1. According to the infra-red spectrum, it is considered that humic acids contain such substituent groups as hydrogen-bonded OH, aromatic and aliphatic CH groups, COOH, C=O, and C=C, and consist of various aromatic and aliphatic compounds including phenols, quinones, ethers, and alcohols.
2. From the pattern of the spectra, humic acids are tentatively classified into four types, i.e. A, B, Rp, and P.
3. The most significant change in the absorption bands of soil humic acids with the progress of humification is the increase in intensity of the bands arising from the oxygen-containing groups, but there seems to exist some evidence indicating the increase of the aromatic nature of humic acids during humification processes.
4. Among artificial humic acids, the humic acids prepared from hydroquinone and from the pigment produced by *Penicillium islandicum* are remarkably similar in shape to the spectrum of the A type, and the one obtained from farmyard manures and glucose to the B type. The infra-red spectra of the other artificial humic acids are comparatively analogous to those of soil humic acids, but some differences are found between them.

26. A new method for determining ferrous iron in paddy soils. Kyoichi KUMADA and Teruo ASAMI (Soil and Plant Food 3, 187–193, 1958)

To find a suitable method for the determination of ferrous iron in paddy soils, the writers conducted a number of experiments using dilute sulfuric or hydrochloric acid solutions, and found that these acids were not adequate, because they dissolved not only ferrous iron but also ferric iron which was reduced to ferrous form by reducing substances extracted simultaneously. A new method with 1N acetate buffer (pH 2.8) as the extractant is considered to be suitable for our purpose.

In addition, preliminary experiment was carried out on the forming process of ferrous iron in paddy soils under waterlogged condition.

27. Fundamental studies on aerobic fermentation. Part 6. Power requirements of aeration and agitation. Koichi YAMADA and Joji TAKAHASHI (Jounr. Agr. Chem. Soc. Japan 32, 344, 1958)

In aerobic culture, oxygen absorption coefficient Kd has great influences on the growth and fermentation products of microorganisms.

The Power consumptions in aerations and agitations which are required to provide aerobic cultures with some values of Kd were determined in three types of laboratory fermentors.

The requirements of the power for agitations were determined by means of torque table, and those of aerations were determined theoretically.

Based on the relations between Kd and total power consumption, (aeration power + agitation power), the most suitable aeration rate and agitator speed for

minimizing the total power consumption which are required to supply an aerobic culture with optimum Kd were considered.

(1) Under the constant agitator speed, the total power consumption decreased accordingly as the air flow increased, up to the linear velocity of 1 cm/sec. On the other hand, the Kd value increased in proportion to the increase of air flow. Therefore, as shown in fig. 1, the most recommendable air flow rate was about 1 cm/sec.

(2) The following empirical formula was obtained, under constant air flow, between Kd and total power consumption per unit volume (Pt/V).

$$Kd = k(Pt/V)^{\alpha}$$

hence, α, k: constant

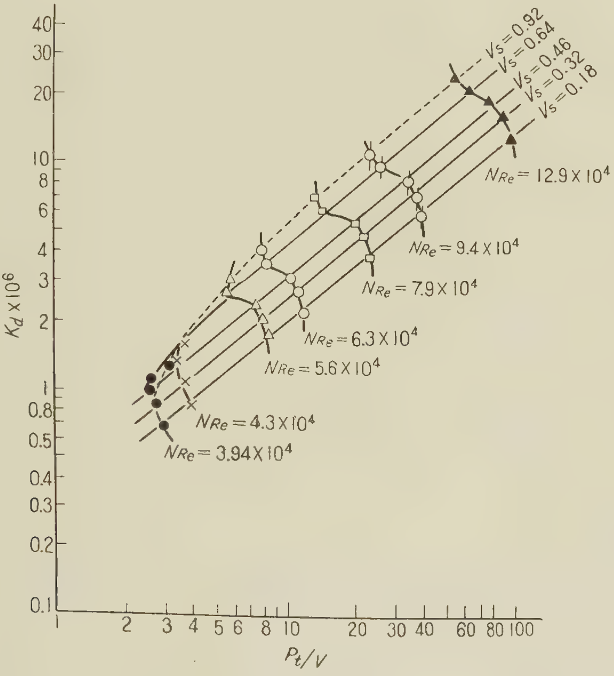


Fig. 1.

28. Dynamic studies on nutrient uptake by crop plants. Part 13. The effect of metabolic inhibitors such as $NaN_3 \cdot NaF \cdot IAA \cdot MA \cdot NaCl \cdot 2\text{-}4\text{-}D \cdot DNP$, particulary NaF an the nutrient uptake by rice plant. Shingo MITSUI and Kikuo KUMAZAWA (Journ. Sci. Soil and Manure, Japan 27, 439–442, 1957)

The effect of several metabolic inhibitors such as sodium azide, sodium fluoride, iodoacetate, malonate, sodium chloride, 2.4-dichlorophenoxy-acetic acid and 2.4-dinitrophenol on the nutrient uptake by rice plants was studied. Utilizing mature rice plants the following results were obtained.

1) 2.4-ditrophenol (ca 10^{-2}M), sodium azide (1×10^{-2}M), sodium mlaonate

(5×10^{-2}M) and sodium iodoacetate (1×10^{-2}M) markedly inhibited root respiration as well as nutrients uptake. Phosphorus and potassium which is probably linked more directly to the metabolic cycle during their absorption processes, were excreted out; ammonium nitrogen, water uptake and respiration were depressed.

2) Sodium chloride (1×10^{-2}M) depressed water uptake especially among other elements. It was discussed that at the first step the high osmotic pressure of culture solution caused damage of plant cells similar to drought followed by the inhibition of metabolism and salts uptake.

3) Sodium fluoride (1×10^{-2}M) remarkably inhibited potassium uptake, but not so much phosphorus, ammonium nitrogen and water uptake, whereas the respiration rather increased.

Further, the inhibition was precisely re-examined with rice seedlings, changing the concentrations from 0 to 10^{-1}M NaF. At lower concentrations of NaF (10^{-4}, 10^{-3} and 10^{-2}M), the uptake of phosphorus, ammonium nitrogen, water and oxygen was not or very slightly decreased; the uptake of potassium decreased almost proportionally to the concentration of NaF. At the highest concentration of NaF (10^{-1} M) potassium and phosphorus were even excreted out, and the uptake of ammonium nitrogen, water and oxygen was remarkably depressed, similar to 10^{-2}M NaN_3. By the use of radioactive phosphorus, it was demonstrated that sodium fluoride inhibited only phosphorus translocation from root to shoot, but it is indifferent to phosphorus accumulation in roots. This can be explained on the assumption that, at the first step of accumulation of phosphorus, its esterification by such pathway as oxidative phosphorylation etc., is required, and the phosphate spliting off catalized by phosphatase etc. precedes its excretion to xylem. By comparatively low concentrations of sodium fluoride, the latter process possibly might have been inhibited and by comparably higher concentrations the former via enolase or succinic acid oxidase inhibition. Potassium uptake is believed to be linked with this dephosphorylation process, by supplying some binding body or bonding energy of potassum.

Part 14. Intake and transformation of $H_3P^{32}O_4$ in the root of wheat seedlings. Shingo MITSUI, Sueo ASO and Kozo ISHIZUKA (Soil and Plant Food, 3, 65–69, 1957 and Journ. Sci. Soil and Manure, Japan 28, 262, 1958)

The turnover of the intaken phosphates within the root of wheat seedlings was traced during a short time interval, (eg. a second or a minute) with the use of radioactive phosphate P^{32} and the technique of radio-autogram of paper chromatography.

In addition to nucleotide-like substances, G-6-P and F-6-P were primarily accumulated within the root and existed relatively in higher concentrations than other organic phosphates. Also, varieties of organic P^{32} compounds increased as time elapsed.

In the exepriments with inhibitors, phlorizin, CH_2ICOOH, NaF and NaCN were used. They showed characteristic and different types of inhibition respectively. It was concluded that the enzymatic connection of phosphate and sugars and also turnover of products are absolutely required for phosphate utilization.

Part 15. Nutrients uptake as influenced by nutritional status of lowland rice. Shingo MITSUI and Kikuo KUMAZAWA (Journ. Sci. Soil and Manure. Japan 28, 265, 1957)

With the help of ordinary water culture technique the following results were obtained.

In the treatment of low N, P, K and Ca the depression of nutrient utpake of the resepctive elements associated with Ca was comparatively low or rather increased.

Moreover, the deression of the utake of P and K in low N level, K and N in olw P level, N in low K level and N, K in low Ca level was comarativeyl remarkable as compared with other nutrient elements.

The results were discussed particularly on the basis of O_2 uptake.

Part 16. Salt effect on phosphate uptake (partly utilizing P^{32})by crop plants. Shingo MITSUI and Kiyoshi TENSHO (Journ. Sci. Soil and Manure. Japan 28, 327–331, 1957)

A series of exepriments have been conducted in order to clarify the relationship between plant-root, soil and fertilizers using neutral salt and phosphates. As the first report of the experiments, the following results were obtained.

1. On the water culture experiments, the root uptake of phosphoric acid was increased by the presence of salts, showing the good agreement with Mattson's hypothesis based on the principle of Donnan membrane equilibria.

It was found, however, that the general effect depending upon the root metabolism, which lowered the pH of nutrient solution, should be taken into consideration as a secondary factor contributing to the salt effect.

2. Similar salt effects were observed on soil fixation and root uptake of phosphoric acid using Tanashi volcanic-ash soil.

Part 17. Salt Effect on phosphate absorption by soils. Shingo MITSUI and Kiyoshi TENSHO (Journ. Sci. Soil and Manure. Japan 28, 345–350, 1957)

The increasing fixation of phosphoric acid in the presence of salts (Salt effect) was studied using Tanashi volcanic-ash soil. The results obtained were as follows.

1. In the alkaline condition, salt effect was predominantly higher, but as the pH decreased it turned to show the negative tendency of the result. This tendency was more significant in case of sulfates in comparison with chlorides.

2. The degree of salt effect was observed in the following order with regard to various kinds of cations which coincided to the lyotrophic series.

$$Na < NH_4 < K, \quad Mg < Ca$$

3. The phosphoric acid fixed due to salt effect could be redissolved by the dilution of salts. (Salt dilution effect)

29. Iron and manganese metabolism in crop plant as influenced by copper. Part 1. Study on the chlorosis of upland rice cultivated on copper affected area and on the copper accumulation in orchard soils. Shingo MITSUI, Kiyoshi TENSHO, Kikuo KUMAZAWA, Tetsuo FUJITA and Jinya YAZAKI (Journ. Sci. Soil and Manure. Japan 28, 505–507, 1958)

Survey on the chlorosis of upland rice cultivated on the copper affected area along the River Watarase (Ōra-gun, Gunma-ken) showed that it is closely connected with the accumulation of copper associated with the application of lime.

Crop and soil samples collected on this area were analysed on pH, Cu, exchange-

able and reducible Mn in the soils and on Cu, Fe, Mn, N content of the crop plants. The result of analysis does notallow any simple interpretation of the ultimate cause of chlorosis, but seems to implicate a much more complicated interaction among Cu, Fe and Mn, which will be elucidated in the further reports.

Incidentally, the chlorosis often occurring in the orchards with long term and heavy Bordeaux spraying was attributed to the heavy accumulation of Cu in the surface soil comparable with Cu mine pollution.

30. Investigations on the radioactive contamination of crop plants as a result of the nuclear detonation. Part 3. Root uptake of fission products. Shingo MITSUI and Kiyoshi TENSHO (Journ. Sci. Soil and Manure. Japan **29**, 71–76, 1958)

1. Wheat seedlings grown on quartz sand with the technique similar to Neubauer were able to accumulate in tops, 3.4%, 2.0%, 0.013% and 0.71% of the applied doses in Sr^{90}, Cs^{134}, Ce^{141} and gross fission products respectively.

Moreover, T/R ratio of radioactivity of those elements showed the same following order as the degree of accumulation in tops.

$$Sr^{90} > Cs^{134} > f.p. \gg Ce^{141}$$

The uptake was in most cases significantly decreased especially in case of Ce^{141} by addition of calcium salts such as $CaCO_3$, silicate slag and monocalcium phosphate into the root medium; Sr^{90} and gross fission products were particularly decreased by the addition of phosphates.

2. Kidney bean and buckwheat were grown on pots containing Tanashi volcanic ash soil added with ca. 0.2 mc of gross fission products.

The concentration of radioactivity in seeds on both dry and ash weight basis was much lower than in other portions.

According to the radiochemical group analysis the radioactivity was predominantly due to strontium ($Sr^{89}+Sr^{90}$), showing the outstanding selective absorption of this element by plant roots.

Part 4. Radioactive contamination of crop plants and soils especially with regard to Sr^{90} from 1956 to 1957. Shingo MITSUI, Kiyoshi TENSHO, Ko-Ling YEH and Katzumi ONO (Journ. Sci. Soil and Manure. Japan **29**, 109–116, 1958)

During the periods from May to July of 1956, several kinds of field crops were collected once a week from three plots near Tokyo, and the gross radio-activities of ashed samples were measured.

The contamination of leaves was highest as compared with stalk, pods and roots. The seasonal change substantially depended on the rain-out activities. Of the samples collected on June 25, the highest value was detected in the leaves of kidney bean and egg plant at about 1300 cpm (3.7 $m\mu c$) per 10 g dry weight.

Moreover, the deconstamination by washing with water, dilute acids and dilute salt solutions was very imperfect. The contamination seemed in most cases to exceed the level of permissible concentration of unknown mixture of radio-isotopes, suggesting the need of further nucleids separation.

A series of strontium radioassay was conducted with crop plants, particularly rice and soils collected in Kanto Province during the period from November 1956

to June 1957.

The exchangeable Sr/100 Ca ratios on weight basis were from 0.65 to 1.05 for paddy soils, with one exceptionally low value of 0.12 for the playground of Tokyo University.

The values for vegetables were from 0.71 to 1.02 showing an outstanding similarity to those of soils. However, the seeds of rice, wheat and beans showed lower values as as from 0.09 to 0.36.

The Sr^{90} concentration in top soils were higher than subsoils especially in case of uncultivated soil. Further, this tendency was more pronounced for Sr^{89} values.

As for the Sr^{90} concentration of crops, in term of S.U., i.e. Strontium Unit or Sunshine Unit defined as Sr^{90} $\mu\mu c/g$ Ca, the most remarkable feature was the high seed activity of rice and wheat, which showed from 81 to 250 for brown rice grown in 1956 and from 120 to 170 for wheat grain grown in 1957 respectively as contrasted with the values from 4.5 to 17 for beans and vegetables. The latter values are approximately in the same order of soils. On the dry weight basis, however, this trend was reversed and the Sr^{90} activity was more remarkable in calcium rich plants such as vegetables, than in cereals.

The S.U. of polished rice was about one tenth of the husks and brans of rice, while the S.U. of wheat flour was about one third of its brans.

It was concluded that the route of contamination of Sr^{90} for calcium rich plants were mainly due to the root uptake, and for calcium poor plants or plant organs the direct or superficial contamination became the main route as compared with the root uptake.

31. The utilization of Ca by crop plants from Ca^{45} lebeled lime nitrogen. Shingo MITSUI, Kiyoshi KURIHARA, Kazuyoshi HAMADA and Akira TERAKAWA (Journ. Sci. Soil and Manure. Japan **29**, 163–166, 1958)

The uptake and utilization of Ca carried in lime nitrogen by a crop plant was traced on the concept that the said calcium would not only serve effectively to neutralize the acidity or improve the physical properties of the soil, but also to help the plant growth as a nutrient.

Two types of soil, were chosen for the present experiment namely, the Tanashi soil low in same. Lime nitrogen labeled with Ca-45 was applied in distinction from the soil Ca. Wheat was grown in small Wagner pots, each being 1/500,000 hectare in surface area. Samples were taken and analyzed according to the periods of elongation, heading and harvest. For measurement of radioactivity, the sample of Ca-oxalate was prepared, and correction was made for self absorption from self absorption curves drawn up in advance. The results of the present experiment can be summarized as follows:

a) The sample plant absorbed Ca of lime nitrogen source appreciably and continuously throughout the entire period of its growth. The absorption of Ca-45, computed in terms of availability, was 0.12-0.89% for the Tanashi soil and 0.30-1.79% for the Takashigahara soil respectively.

The fact that the uptake of Ca-45 was sustained at practically the same level up to the harvest time is suggestive that calcium will have a very satisfactory residual effect on the second crop.

b) The results of measurement at each section of the plant body and the radio-autographs show that unlike phosphorus and other fertilizer elements, Ca was absorbed and distributed substantially uniformly throughout the plant body, excepting the metabolically active new organs where a slightly larger amount of Ca was checked.

c) In the Takashigahara soil plot deficiency in exchangeable, Ca, the absorption of Ca-45 was remarkable (about double the rate in Tanashi plot). The specific activity of Ca-45 absorbed by the crop plant tended to be more pronounced as the growth advanced.

d) The percentage of Ca from lime nitrogen source included in the total plant calcium at the harvest time accounted for 16.7 per cent for the Tanashi soil plot and 59.2 per cent for the Takashigara soil plot. It attracted attention that the ears and the leaves and stems of the plant cultivated in the latter plot recorded 85.7 and 70.4 per cent respectively, showing that the greater part of calcium absorbed in the said portions consisted of calcium from lime nitrogen. It is considered that lime nitrogen made great contributions to acid soils short of calcium not only as an agent to neutralize the acidity but also as an important source of calcium which will also serve as a nutrient to promote the growth and yield of the crop plant.

Since the nutrifying value of calcium, a sub-component of lime nitrogen, varies with the kind of soil, the variety of crops, climatic conditions and other various factors involved, further study will be necessary to reach a more definite conclusion. However, the present experiment can be said to have proved quite clearly that the tracer technique possesses great advantage in developing such types of experimental study.

32. Studies on with reference to the green manure for rice field. Katsura TSUZIMURA, Fumiyo IKEDA and Kazuo TSUKAMOTO (Journ. Sci. Soil and Manure. Japan 28, 275, 1957)

Azolla japonica grows in spring before rice transplantation and fixes atomospheric nitrogen. The plant is promising as a green manure crop for rice field. The growth responded markedly to the dressing of superphosphate of lime. The maximum yield in the small scale experiment was 3.2 g nitrogen from 2.5 m^2 which corresponded to 128 kg per hectare. The growth in the nutrient solution without nitrogen was excellent, although a small amount of nitrate or ammonia accelerated the growth.

The symbiotic blue-green algae was *Anabaena azollae*, Strasb., identical with the symbiotic organism of Europian *Azolla*.

33. Fe-EDTA chelate for water culture. Katsura TSUJIMURA and Fumiyo IKEDA (Journ. Sci. Soil and Manure. Japan **27**, 479–480, 1957)

Ethylene diamine tetra-acetic acid prevented the precipitation of iron from water culture solution at pH 6 or more acid condition. But it was not effective at pH 8.

EDTA chelate of iron was convenient for the water culture of rape, wheat and rice.

34. Leaching of sulfate and calcium as influenced by particle size of fertilizers. Shingo MITSUI, Masaaki MORIYAMA and Kiyoshi TENSHO (Journ. Sci. Soil and Manure. Japan **27**, 481–484, 1957)

It is very important to clarify the movement of sulfates in the paddy soil, especial-

ly with regard to the physiological damage of paddy root.

A pot lysimeter experiment was conducted to measure the sulfate and calcium leaching out from an alluvial paddy soil, in relation to the particle size of fertilisers including both ions and its complementary ions.

The results showed that the amounts of sulfate and calcium in the leachates had similar trend of seasonal change except for sulfate of non-fertiliser pots which was exceptionaly low in the total amount. This means that the greater part of the leached sulfate was derived from the applied fertiliser, and the leached calcium was largely due to the native soil calcium via base exchange.

Further, the leached sulfate showed little differences through granulation the leached calcium, on the contrary, decreased as the particle size of fertilisers increased.

This may possibly be explained according to the hypothesis that the path-way of leached ions from larger granulated fertilisers is restricted to the smaller part of soil columns as compared with powdered fertilisers, thus restricting base exchange and sulfide contamination with a limited area of the furrow slice.

35. The intake and utilization of carbon by plant roots from C^{14}-labeled urea. Part 1. The determination of radioactive carbon of plant materials and a preliminary seedling experiment utilizing C^{14}-labeled urea. Shingo MITSUI and Kiyoshi KURIHARA (Soil and Plant Food, 3, 59–64, 1957 and Journ. Sci. Soil and Manure, Japan 28, 473–443, 1958)

The intake of carbon, utilizing C^{14}-labeled urea was studied with respect to lowland rice grown on waterlogged soil and wheat grown on upland soil. First, the reliability of the radioassay of the C^{14} taken in the plant body was examined. A slight improvement of the common method of sampling, wet combustion, mounting $BaC^{14}0_3$ and radioassay was necessary to increase reliability. The C^{14} intake by rice seedlings was about 6.38 in term of percentage of availability, as compared with extraordinary low values obtained with wheat seedlings. The absorption via plant roots of urea or ammonium carbonate decomposed from urea continued 10 days at 20-25°C., and the soil added was minimized so far as the natural decomposition speed of urea was maintained.

The reason for the outstanding difference of intake C^{14} with respect to lowland rice versus wheat, and to waterlogged versus upland soil condition was discussed .

36. Studies on isoamylase. Part 9. Purification of isoamylase. Tsuneo KOBAYASHI (Journ. Agr. Chem. Soc. Japan 31, 865–867, 1957)

Isoamylase from brewer's yeast is purified after the stability of the enzyme is examined against pH, temperature, and various procedures of enzyme purification. The enzyme is fairly stable at pH 6-9, and 0°C., and is rapidly inactivated below or above that pH range, or at temeperatures above 25°C. It is inactivated by a treatment with thanol or acetone at room temperature, but the enzyme can be precipitated without appreciable loss in activity with ammonium sulfate, 30% acetone, or 30% ethanol at a temperature below −5°C. Adsorption and elution method on diatomaceous earth, kaolin, or Japanese acid clay can be also applied to the purification, but the recovery of the enzyme activity is not high.

The enzyme is successfully purified in an electrophoretic separation in a starch-supporting medium. The enzyme precipitated with ammonium sulfate or acetone is dissolved in 0.1 *N* veronal buffer of pH 8.6, and subjected to electrophoresis in a starch block placed in a cold room. After 24 hours of electrophoresis, the starch block is cut into segments of 1 cm. width, and each segments are extracted with water. The specific activity of the enzyme fractions thus obtained is raised to about five to ten times of that of the original solution. Purified isoamylase preparations are free from amylase, phosphorylase, maltase, and isomaltase.

Part 10. The substrate specificity of the isoamylase. Tsuneo KOBAYASHI (Journ. Agr. Chem. Soc. Japan **31**, 868–872, 1957)

The action of purified isoamylase was studied on several oligosaccharides containing α-1, 6-glucosidic linkage. The enzyme acted on the branching point in α-limit dextrin of the mean polymerization degree of 5, which was prepared by the action of crystalline Takaamylase A on starch. But the isoamylase was without action on isomaltose, isomaltotriose (dextrantriose), and panose. Results on isomaltose were not consistent with those of Manners and Maung (Chem. & Ind. 1955, 950), and the discrepancy was ascribed to the difficulty in removing isomaltase from isoamylase preparations.

Structural requirements of oligosaccharides for susceptibility to the isoamylase action were discussed, and following conclusions were drawn: the presence of additional α-1,4-glucosidic linkage(s) adjacent to the 1,6-linkage was required for the enzyme action; but a 1,4-linked glucose residue attached to the C_1 of the glucosidic residue bearing a branch link in panose did not satisfy this requirement; the smallest substrate for the isoamylase would be of the structure, $G_1-\overset{\overset{G_1}{|}}{G_1^6}-_4(G)$, where G represents a glucosidic residue.

The actions of isoamylase, amylo-1,6-glucosidase, oligo-1,6-glucosidase, dextranase, and R-enzyme were compared and differences among them were discussed. A possible identity of the actions of isoamylase and R-enzyme was mentioned.

37. Studies on dextran. Part 5. Dextran-destroying enzyme of molds. (2) Mono-enzymic nature of mold dextranase and the activation energy in the hydrolysis of α-1, 6-glucosidec linkages. Tetsuo KOBAYASHI (Journ. Agr. Chem. Soc. Japan **31**, 494–496, 1957)

Several features are described on the action of dextranase of *Penicillium funiculosum*. Decrease in the solution viscosity and the formation of reducing sugars are observed in the dextranase action on dextran. These two activities of dextranase cannot be separated by the fractionation of the enzyme, and their ratio of activity remains nearly constant in acid- and heat-inactivation of the enzyme. It is concluded that the two actions are catalyzed by a single enzyme.

The apparent activation energy in the enzymic hydrolysis of α-1,6-glucosidic linkages is estimated from the rates of dextran hydrolysis at various temperatures. A value, 17,400 cal./mole, is obtained from data between 15°C, and 30°C. The value is about 54% of that observed in the acid hydrolysis of dextran.

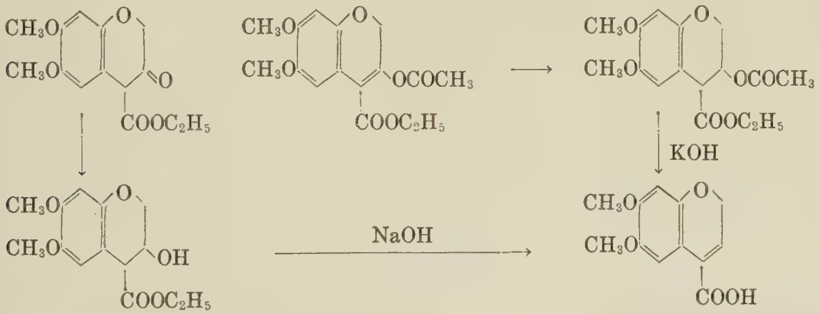
Part 6. Dextran-destroying enzyme of molds. (3) Michaelis constant for dextran-dextranase system and its variation with molecular weight of the substrate. Tsuneo KOBAYASHI (Journ. Agr. Chem. Soc. Japan 31, 497–500, 1957)

The dextran hydrolysis by mold dextranase does not proceed completely, and an apparent limit is reached at a degree of hydrolysis above 60%. The limit is varied with the amount of the enzyme used. The Michaelis constant is estimated for dextran of several molecular sizes to elucidate this incomplete reaction. Values of Ks% (Michaelis constant expressed in % concentration of the substrate) obtained are 4.2×10^{-2}, 1.9×10^{-1}, and 2.2×10^{-1}, respectively, for dextran of mean moleculat weight of 66,000, 43,000, and 17,000. Ks (Michaelis constant expressed in molar concentration of the substrate) are 6.4×10^{-6}, 4.4×10^{-5}, and 1.3×10^{-4} M., respectively, for three specimens of dextran.

The results indicate that the affinity of dextranase towards the substrate is much decreased with the decrease in molecular weight of dextran. The decrease in affinity is sufficient to explain the incomplete dextran hydrolysis.

38. Syntheses and configurational elucidations of rotenoids. Part 1. Contributions to the synthesis of toxicaric acid. Masateru MIYANO and Masanao MATSUI (Bull. Chem. Soc. Japan 31, 267, 1958)

The improved synthesis of toxicaric acid (I) which is a decomposition product of several rotenoids and seems to be a probable starting material for the total synthesis of natural rotenoids is carried out via the next two routes:

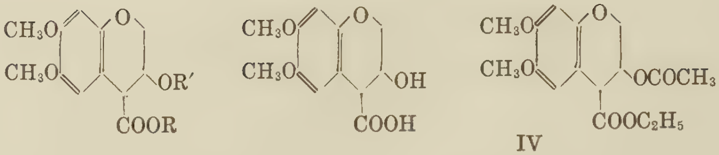


When treated with boiling alkali no intermediate hydroxyacid (hydroxynetoric acid) was isolated. The authors believe the method described here is the best for preparation of the acid.

Several new compounds related to toxicaric acid are also synthesized and characterized. Infra-red and ultra-violet data are given.

Part 2. On the synthesis and the steric configuration of dl-hydroxynetoric acid. Masateru MIYANO and Masanao MATSUI (Bull. Chem. Soc. Japan 31, 271, 1958)

dl-cis And dl-trans-hydroxynetoric acids (I, II) which might serve as key compounds for the determination of the steric configuration of natural rotenoids and at the same time are probable starting materials for the synthesis of natural rotenoids are obtained by very mild alkaline hydrolysis of the dl-cis ethyl



IV

I: R=R′=H, m.p. 161°   II: R=H, m.p. 156–7°

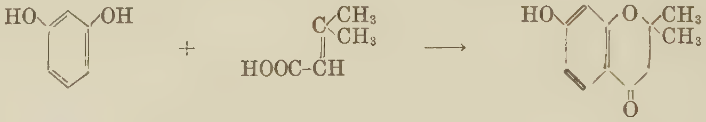
III: $R=C_2H_5$, R′=H   V: $R=C_2H_5$, $R=COCH_3$

ester (III). I is partly isomerized to II by basic catalyst. When treated with hot alkali both I and II are converted into toxicaric acid. The catalytic hydrogenation of the enol-acetate (IV in the presence of palladium-charcoal affords a Acetoxynetoric acid ester (V) which is clearly the cis-compound. The mild acetylation of the ethyl ester (III) of which no configuration is determined hitherto also affords the same product (V). Hence the ester (III) must have cis-configuration, the main product of the mild hydrolysis of (III) is I which must then be cis-form. Other data are all consistent with the above assignment.

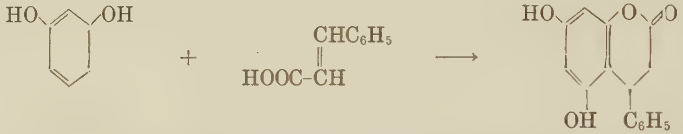
Either of the synthetic hydroxynetoric acids does not coincide with the dl-hydroxynetoric acid (m.p. 189°) isolated from a natural compound.

Part 3. On the reaction of α,β-unsaturated acids with resorcin or phloroglucin. A new synthesis of chromanone ring. An attempted synthesis of rotenone. Masateru Miyano and Masanao Matsui (Bull. Chem. Soc. Japan 31, 397, 1958)

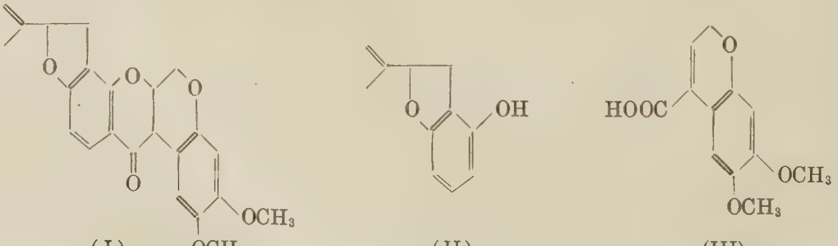
β,β-Dimethylacrylic acid or crotonic acid when heated with resorcin or phloroglucin in antimon trichloride afford chromanone-compounds.



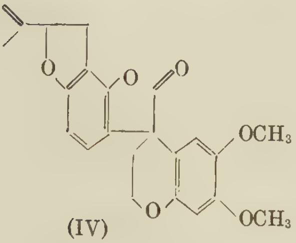
To the contrary, cinnamic acid affords dihydrocoumarin-compound.



The attempted synthesis of rotenone (I) by the condensation of tubanol (II) and toxicaric acid (III) in the presence of antimon trichloride failed. The main product is a β,γ-unsaturated-γ-lactone (IV) which has no insecticidal activity.



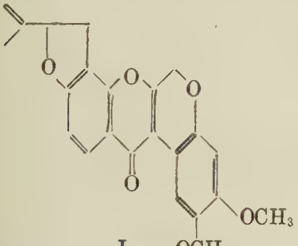
(I)   (II)   (III)



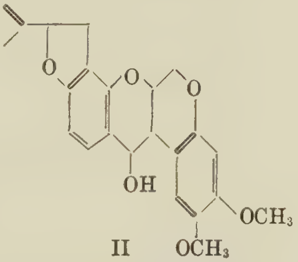
(IV)

Part 4. Partial synthesis of the natural rotenone. Masateru MIYANO and Masanao MATSUI (Bull. Bhem. Soc. Japan **22**, 128, 1958)

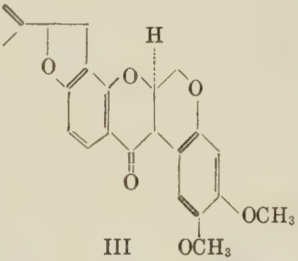
Reduction of dehydrorotenone (I) with sodium borohydride affords a new compound (II) which is named rotenol. Oppenauer oxidation of II



I

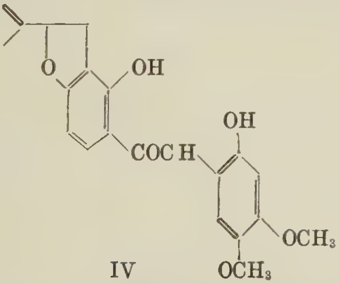


II

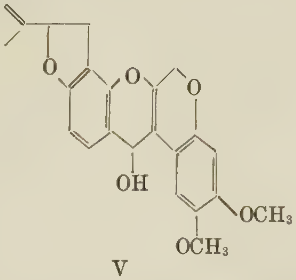


III

affords the rotenone which is identical with the natural product in all respects. From these reactions the trans-configuration of the natural rotenone is strongly suggested. As the synthesis of dehydrorotenone



IV



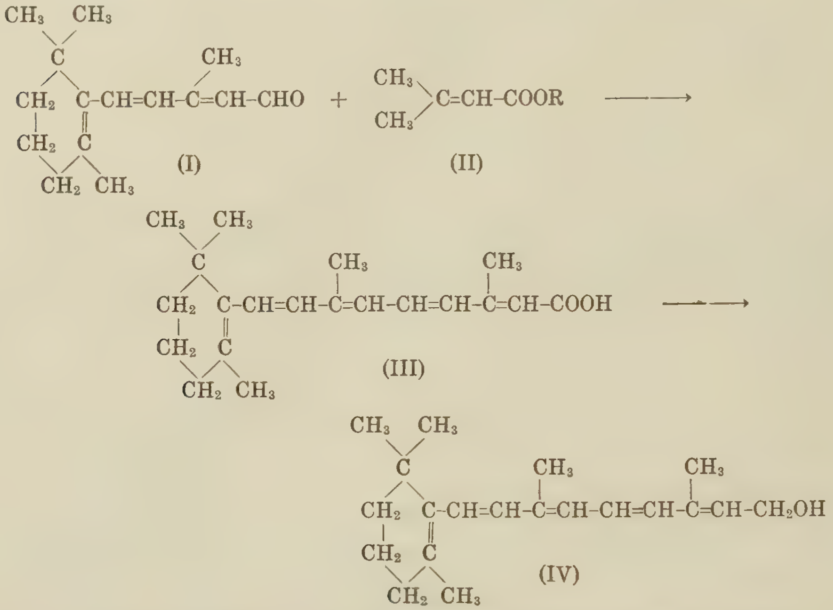
V

from derritol (IV) was already described in literature, our result means the synthesis of rotenone from derritol. Another approach to the rotenone synthesis is also described which consists in lithium aluminium hydride reduction of dehydrorotenone to an unsaturated alcohol (V) following rearrangement by active alumina.

39. A new synthetic method for vitamin A. Masanao MATSUI, Kyôhei YAMASHITA, Masateru MIYANO, Seiichi KITAMURA, Shigeru OKANO, Akio KOBAYASHI, Tomiichi SATÔ and Ryûzo MIKAMI (Proceed. Japan Academy **34** [4], 220, 1958)

The authors, in studying on the condensation of ester of senecioic acid (II) with aldehydes, found that the ester condenses readily in the presence of alkali amide with

trans-β-ionylideneacetaldehyde (I) to produce vitamin A acid (III).



Starting material (I) was obtained by two methods: 1) MnO_2 oxidation of trans-β-ionylidene-ethanol which was derived from trans-β-ionylidene-acetic acid by $LiAlH_4$ reduction and 2) MnO_2 oxidation of β-ionylidene-ethylamine which was obtained by $LiAlH_4$ reduction of β-ionylidene-acetonitrile.

The either solution of 1 mole of (I) and 1.5 mole of (II) was added into 2 mole of potassium amide in liquid ammonia and mixture was stirred for 72 hours. After evaporation, saponification, extraction and acidification, crude (III) precipitated. The yield of crude acid was 90%, it was recrystallized from ethanol and 60% yield of pure all-trans-vitamin A acid, m.p. 181-2°, λmax 340 mμ, εmax 44,500 was obtained. The structure was confirmed by comparing with authentic specimen prepared by the method of Robeson et. al.

The methyl ester of (III), m.p. 56-7° and 72-3°, λmax 355 mμ, εmax 42,800 was obtained from (III) and diazomethane. $LiAlH_4$ reduction of the ester afforded theoretical yield of all-trans-vitamin A (IV), λmax 325 mμ, εmax 49,600. Acetate, m.p. 57-8°, λmax 326 mμ, εmax 48,000 was prepared from (IV) and acetyl chloride in the presence of pyridine.

40. Infrared spectrophotometric determination of traces of parathion in water.
Yusuke Sumiki and Akira Matsuyama (Bull. Agr. Soc. Japan **21**, 329, 1957)

The third method which permits to carry out the infrared analysis, has been designed in order to establish a more specific method for parathion determination. The Method 3 proposed for parathion determination is based on the solvent extraction, chromatography using heavy paper and infrared spectrophotometry. This method

is also applicable to other insecticides and the sample can be treated on a scale of 100 mg or more, if necessary.

Procedure: The steps of this method can be summarized as follows:

(1) Collection of parathion by liquid-liquid extraction. Parathion in the water sample can be quantitatively extracted into 2-4 1 of the solvent (equal volume mixture of benzene and hexane) by passing 200 1 of the water sample through the extractor at the rate of 100-120 1 per hour.

(2) Concentration of extracted layer, using an agitating evaporator.

(3) Isolation by paper chromatography. Two separate chromatographic systems by descending method are employed in conjuntion, using the thicker paper and nonaquous solvent systems (The first: ethanol-nitromethane or ethanol only as a mobile solvent, soybean oil as a stationary solvent. The second: n-hexane as a mobile solvent, ethylenechlorohydrine-methanol as a stationary solvent).

(4) Elution from paper chromatogram by ether.

(5) Infrared spectrophotometry. A carbon disulfide solution can be used for qualitative identification in the 2- to 15-micron region except the 6.2- to 7.0-micron region (thickness at 0.1 mm) and also for quantitative determination by measuring the absorption at 7.37, 9.75 and 10.90 microns.

(6) Quantitative measurement of amounts of parathion.

The recovery in the anlysis of the well-water sample by this method is 94%.

41. Biochemical studies on "Bakanae" fungus. Part 41. Chemical structure of gibberellins. X. Nobutaka TAKAHASHI, Yasuo SETA, Hiroshi KITAMURA and Yusuke SUMIKI (Bull. Agr. Chem. Soc. Japan **21**, 327, 1957)

We proposed the assumption concerning the structural relation between gibberellin A, and gibberellic acid—gibberellic acid has one more double bond in some position of gibberellin A_1 structure—in previous paper. Further evidences were obtained which make sure of this assumption.

On hydrogenation of gibberellic acid, four kinds of products were separated.

a) dihydrogibberellin A_1 in 40% yield.

b) stereoisomer of gibberellin A_1, dp. 268-70°.

c) hydrogenolysis product, $C_{19}H_{28}O_6$, dp. 290-5°, dibasic acid.

d) hydrogenolysis product, $C_{19}H_{28}O_6\frac{1}{2}H_2O$, mp. 184°, dibasic acid.

When gibberellin A_1 and gibberellic acid methyl ester are treated with excess of $LiAlH_4$ in boiling tetrahydrofuran, $C_{19}H_{30}O_5$, mp. 208-10° (A_1Li) from gibberellin A_1 methyl ester and $C_{19}H_{28}O_5$, mp. 208-10° (G. acid Li) from gibberellic acid methyl ester were obtained. On hydrogenation, G. acid Li was converted to A_1Li.

As A_1Li and G. acid Li do not consume periodate, we now have reached a different conclusions concerning the structure of A ring which was proposed by Cross et al.. The consumption of periodate by alkali treated gibberellin A_1, dihydrogibberellin A_1, isogibberellin A_1 and gibberellic acid has been reported.

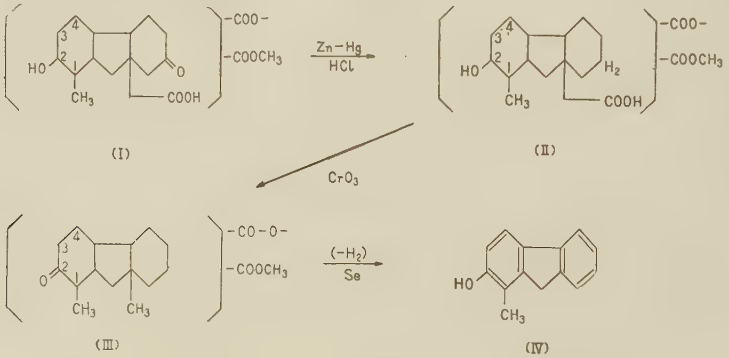
Part 42. A new gibberellin, gibberellin A_4. Nobutaka TAKAHASHI, Yasuo SETA, Hiroshi KITAMURA and Yusuke SUMIKI (Bull. Agr. Chem. Soc. Japan **21**, 396, 1957)

A new gibberellin, $C_{19}H_{24}O_5$, dp. 222°, which was named gibberellin A_4, was isolated from culture filtrate of Gibberella Fujikuroi. Gibberellin A_4 has one

hydroxyl, one lactone, one carboxyl and one exocyclic methylene group. It shows the characteristic physiological activity in about the same degree as gibberellin A_1 by our testing method using rice seedlings, but it inhibits the growth of roots evidently.

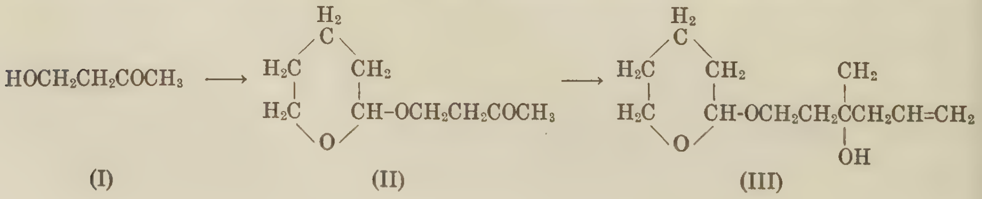
Part 43. Chemical structure of gibberellins. Part XI. Yasuo SETA, Nobutaka TAKAHASHI, Hiroshi KITAMURA, Makoto TAKAI, Saburo TAMURA and Yusuke SUMIKI (Bull. Agr. Chem. Soc. Japan **22**, 62, 1958)

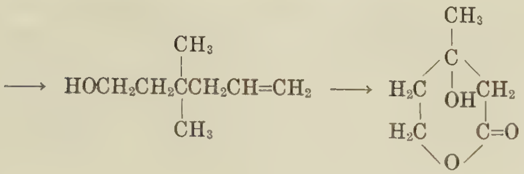
The ozonolysis product of gibberellin A_1 methyl ester, $C_{19}H_{24}O_8$ (I), gives, when reduced by Clemmensen's method with amalgamated Zinc and hydrochloric acid, a desoxo product, $C_{19}H_{26}O_7$ (II). The secondary hydroxyl group of this substance is oxidised by CrO_3, with simultaneous decarboxylation, giving a keto-ester $C_{18}H_{24}O_5$ (III). When this substance is dehydrogenated with selenium, 1-methyl-2-hydroxy fluorene (IV) is obtained. From this fact, the position of the secondary hydroxyl group of gibberellin A_1 is proved to be C-2 of its carbon skeleton.



42. A new synthesis of D,L-β-hydroxy-β-methyl-δ-valerolactone. Saburo TAMURA and Makoto TAKAI (Bull. Agr. Chem. Soc. Japan **21**, 260, 1957)

1-Hydroxy-3-butanone (I), b.p. 65–68°/8 mm, prepared from acetone and paraformaldehyde, was treated with 2,3-dihydropyrane containing a few drops of concentrated hydrochloric acid to give 2-(2-acetoethoxy)-tetrahydropyrane (II), b.p. 97–98°/5 mm, in a 77% yield. When this tetrahydropyranly ether and allylbromide were added to Mg in dry ether, 2-(3-allyl-3-hydroxybutoxy)-tetrahydropyrane (III), b.p. 103–104°/2 mm, was obtained in a 55% yield. III was refluxed gently with dilute hydrochloric acid to afford 3-allyl-1,3-butanediol (IV), b.p. 102–104°/5 mm, in a 77% yield. IV was ozonized in ethyl acetate and the resulting ozonide converted directly into β-hydroxy-β-methyl-δ-valerolactone (V) with





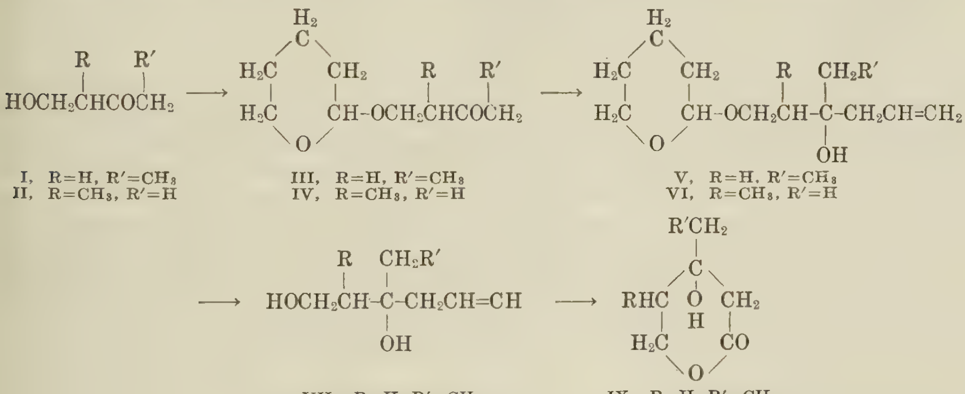
(IV) (V)

hydrogen peroxide in acid solution. Thus, the valerolactone, b.p. 148–151°/5 mm, was obtained as a viscous syrup in a 51% yield. The infra red spectrum of this substance in liquid film is consistent with the structure V, having characteristic bands at 1730 cm^{-1} (δ-lactone) and 3448 cm^{-1} (hydroxyl). It also showed biological activity toward *Lact. heterohiochii.*

43. Synthesis of some homologs of D,L-β-hydroxy-β-methyl-δ-valerolactone. Saburo TAMURA and Makoto TAKAI (Bull. Agr. Chem. Soc. Japan **21**, 394, 1957)

In order to find a new antimetabolite for lactobacilli, some homologs of β-hydroxy-β-methyl-δ-valerolactone (XI) were synthesized. β-Ethyl-β-hydroxy-δ-valerolactone (IX) and β,γ-dimethyl-β-hydroxy-δ-valerolactone (X) were prepared respectively by the similar route as that for XI described in the previous paper. The boiling points of the compounds (I-X) are given below in Table I.

α,β-Dimethyl-β-hydroxy-δ-valerolactone (XIV) was synthesized by the following method. The Reformatsky reaction between 4-acetoxy-2-butanone (XII) and ethyl α-bromopropionate gave ethyl 5-acetoxy-2,3-dimethyl-3-hydroxypentanoate (XIII),



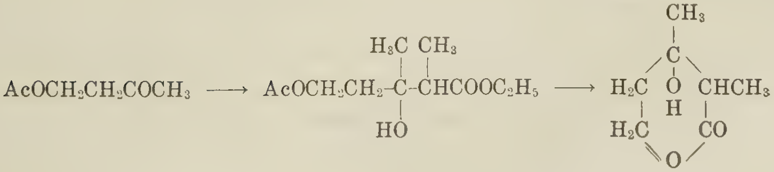
I, R=H, R′=CH_3
II, R=CH_3, R′=H

III, R=H, R′=CH_3
IV, R=CH_3, R′=H

V, R=H, R′=CH_3
VI, R=CH_3, R′=H

VII, R=H, R′=CH_3
VIII, R=CH_3, R′=H

IX, R=H, R′=CH_3
X, R=CH_3, R′=H
XI, R=H, R′=H



(XII) (XIII) (XIV)

Table 1. Boiling Points of the Compounds.

Compd.	b.p., C°	Compd.	b.p., C°
I	71– 72 (7 mm.)	VI	124–127 (5 mm.)
II	77– 89 (20 mm.)	VII	104–106 (4 mm.)
III	93– 95 (3 mm.)	VIII	115–117 (7 mm.)
IV	96– 97 (5 mm.)	IX	130–134 (2 mm.)
V	111–113 (5 mm.)	X	133–137 (2 mm.)

b.p. 125-129°/2 mm. XIII was hydrolyzed with sodium hydroxide in ethanol-water and acidified. XIV was then taken up in chloroform and distilled at 140°/2 mm.

44. The biological activity of some homologs of mevalonic acid. Saburo TAMURA, Gakuzo TAMURA, Makoto TAKAI, Seizi NAKAMURA and Teruo SHIRO (Bull. Agr. Chem. Soc. Japan 22, 202, 1958)

The biological activity of some homologs of mevalonic acid (MVA), β-hydroxy-β-methyl-δ-valerolactone was tested. The compounds tested were as follows. β-ethyl-β-hydroxy-δ-valerolactone, α,β-dimethyl-β-hydroxy-δ-valerolactone (I), β,γ-di-methyl-β-hydroxy-δ-valerolactone, α-thyl-β-hydroxy-β-methyl-δ valerolactone (II), b.p. 136°/3 mm, β-hydroxy-α,α,β-trimethyl-δ-valerolactone, b.p. 145-147°/3 mm, and α-n-butyl-β-hydroxy-β-methyl-δ-valerolactone, b.p. 151-155°/1 mm. Each of the six homologs did not show activity as a growth factor for *Lactobacillus heterohiochii* when added to the broth medium in place of MVA. This fact indirectly suggests that MVA must be a precursor for the isoprene unit. When these compounds were added at the concentration of 100 γ/ml to the medium containing 1 γ/ml of DL-MVA, I or II completely inhibited the growth of the above lactobaccillus while the others showed no inhibition. The minimal concentration of DL-MVA necessary to support growth in the presence of 100 γ/ml of I or II is 4 γ/ml or 3 γ/ml respectively. From these results it may be concluded that only the MVA homologs which have a small alkyl group such as the methyl or ethyl group on their α-carbon atoms can be incorporated into an isoprenoid-biosynthetic systems thus competitively inhibiting the utilization of MVA. At any concentrations, I and II could not inhibit the growth of the lactobacillus which did not require MVA. Moreover, even at the concentration of 100 γ/ml, I or II did not inhibit the growth of *Bacillus subtilis* P. C. I. 219, *Escherichia coli, Staphylococcus aureus* 209 P, *Corynebacterium xeroces, Sarcina lutea, Candida albicans, Tolura utilis* and *Saccharomyces cerevisiae.*

45. Antitumor activities of oximes (II). Kazuhiko TAKAMIYA and Yasuke SUMIKI (GANN, 48, 437–439, 1957)

Antitumor activities of oximes and related chelate-compounds, dimethylglyoximo copper, are described. As for the activities, transoximation between oximes and α-ketoglutaric acid were also examined.

46. Studies on metabolic products of Hypochnus sasakii Shirai. Part 1. Isolation of p-hydroxyphenylacetic acid and its physiological activity. Yu-Shih CHEN (Bull. Agr. Chem. Soc. Japan 22, 136–142, 1958)

Sheath blight (Mongare-byô) of rice plant, caused by Hypochnus sasakii Shirai (=Pellicularia sasakii, Corticium sasakii) is a fungal disease widely spread in East Asia. In Japan, it is one of the most serious diseases of the rice plant.

The characteristic large greenish-grey lesion and its effect on the physiology of rice plant attracted the attention of the author and led to the assumption that the pathological effect may be involved wih a certain toxic metabolic product of the pathogene, therefore, the author's attempts were made to isolate the biologically active substance.

The pathogene was inoculated for about 50 days at 27-29° in a modification of the Richard sol'n.

By means of carbon adsorption, solvent extraction and purification with silica-gel partition chromatography, a physiological substance, $C_8H_8O_3$, melting at 148-149°C was obtained from the above culture broth filtrate.

This substance gave, monomethyl-ether, $C_9H_{10}O_3$, m.p., 87°C, monoacetate, $C_{10}H_{10}O_4$, m.p., 108-109°C, and p-hydroxybenzoic acid, $C_7H_6O_3$, m.p., 214°C, by alkali fusion at 250-280°C.

Upon permanganate oxidation of the methyl ether of this substance, p-methoxy benzoic acid, $C_8H_8O_3$, m.p., 178°C was obtained.

The ultraviolet spectrum in distilled water showed absorption peaks at λ max. 223 mμ (ϵ 7500) and λ max. 275 mμ (ϵ' 1600), while in ethanol it showed at λ max. 232 mμ (ϵ 4500) and λ max. 278 mμ (ϵ' 1800).

However, at the alkaline side they shifted to longer wave lengths and increased their molecular extinctions. The infrared spectrum as Nujol-mull showed characteristic bands of -OH at 3240 and 1215, that of -COOH at 2700, 1700 and 1410, that of aromatic ring at 1610, 1600 and 1520, and that of aromatic 1:4 disubstitution at 790 cm^{-1}.

From the results of ultraviolet and infrared absorption spectara, existence of the phenolic hydroxy group was presumed.

From these evidences, the metabolic product with m.p., 148-149°C is identical with p-hydroxyphenylacetic acid, and identity is confirmed by mixed melting point and accordance of infrared spectrum of the synthetic p-hydroxyphenylacetic acid.

p-Hydroxyphenylacetic acid induced wilt of soybean plant and retarded the growth of rice seedlings at concentrations up to 1:20,000. The O_2 uptake in roots decreased in proportion to reduction of dry weight of rice seedlings.

The author also proved by the application of paper-chromatography and ultraviolet spectrum that, p-hydroxyphenylacetic acid contained in the rice plant is attacked by Sheath blight.

47. Studies on browning reactions between sugars and amino compounds. Part 3. Identification of red pigments and furfural produced from aromatic amine-N-xylosides. Hiromichi KATO (Bull. Agr. Chem. Soc. Japan 22, 85, 1958)

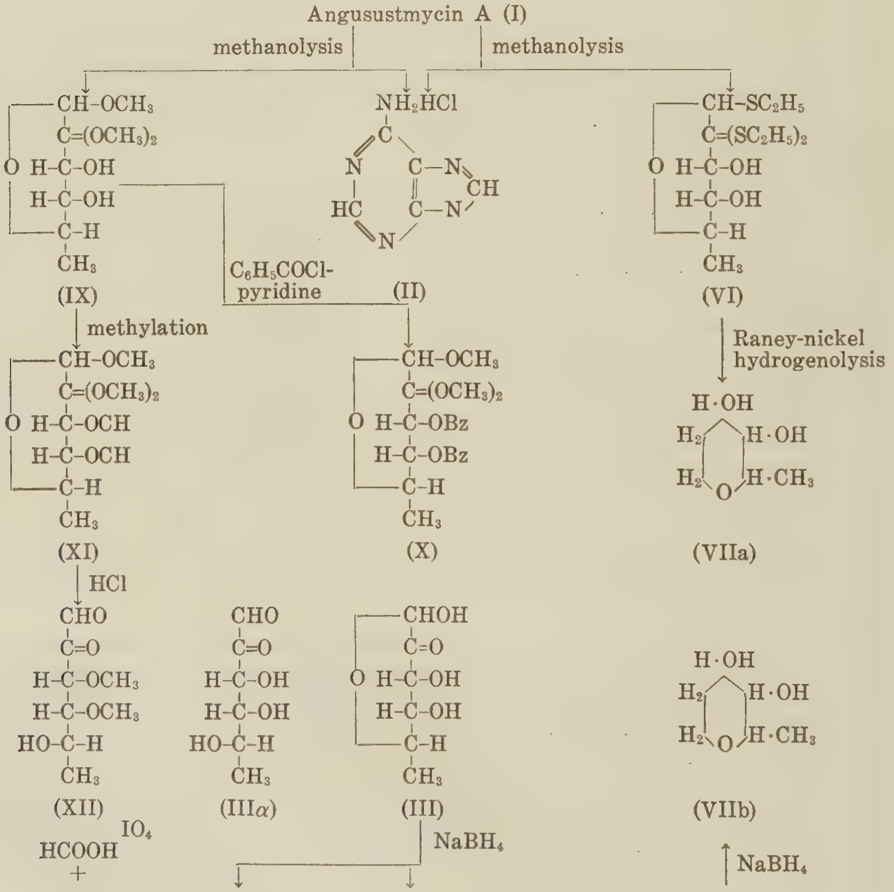
Aromatic amine-N-xylosides were found to roduce both melanoidins and red pigments in methanol solution acidified with hydrogen chloride at 25°. In the hope of elucidation of the mechanism of melanoidin formation between sugars and amino compounds, this red pigments were investigated in this paper. From N-D-xylosyl-p-aminobenzoic acid, 1-p-carboxyphenylimino-5-p-carboxyphenylamino-2-hydroxypenta-

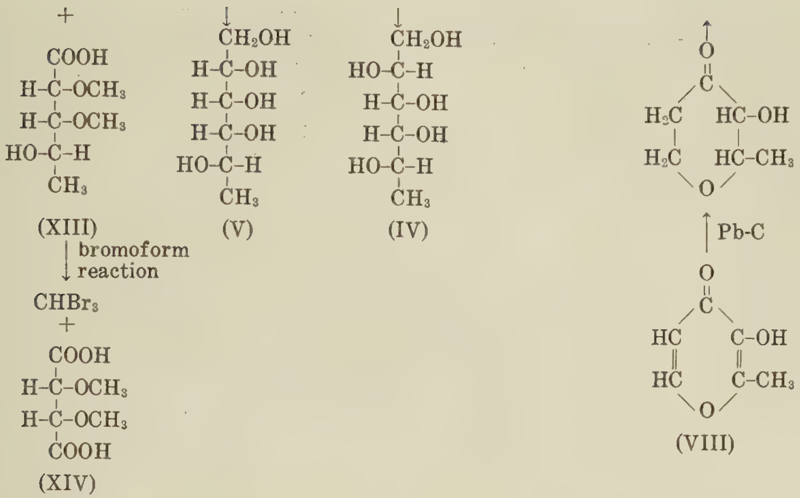
2, 4-diene hydrochloride ($C_{19}H_{19}N_2O_6Cl$, m.p. 221°) and from N-D-xylosylaniline, 1-phenylimino-5-phenylamino-2-hydroxypenta-2, 4-diene hydrochloride ($C_{17}H_{19}N_2O_6Cl$, m.p. 171°) were isolated and identified respectively (theoretical yield, ca. 7%. And further, furfural formed from N-D-xylosyl-PABA or N-D-xylosylaniline under the same condition was identified as 2, 4-dinitrophenylhydrazones (theoretical yield, ca. 20%) of which anti-form (m.p. 225°) and syn-form (m.p. 203°) were clearly separated by adsorption chromatography with alumina.

48. Studies on Augustmycins. Part 5. The structure of angustose. Hsü YÜNTSEN (Journ. Antibiotics. ser. A, 11, 77, 1958)

Angustose, $C_6H_{10}O_5$, a sugar moiety of augustmycin A was elucidated to possess the structure of 2-keto-L-fucopyranose, (III), by the following experimental scheme.

The identification of this particular sugar in natural product may be the first case.

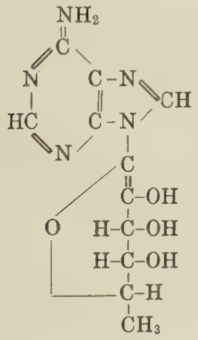




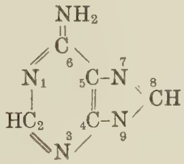
Part 6. **Chemical structureof angustmycin A.** Hsu YÜNTSEN (Journ. Antibiotics Ser. A, **11**, 79, 1958)

The structure of angustmycin A is elucidated as 6-amino-9-(I-1, 2-fucopyraseenyl)-purine (I) by the following experimental data.

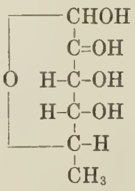
1. On mild hydrolysis, angustmycin A was cleaved into two components which were characterized and identified as adenine (II) and angustose (III) respectively.
2. Angustmycin A shows only one uv. absorption peak at 260 mμ through the range of 220 mμ to 400 mμ. This fact is consistent to the conclusion that the structure of angustmycin A must be adenine nucleoside of common type in which angustose attaches to N_9 of purine nucleus.
3. The fact that angustmycin A consumes two moles of periodate afford to the conclusion of pyranose ring structure for sugar moiety.



(I)



(II)



(III)

49. Studies on the nutritive value of grass proteins. Part 8. The effect of drying methods upon the digestibility of soy bean leaf protein. (B). On the nutritive value of the green leaf of soy bean. Makoto KANDATSU and Tadahiko YASUI (Journ. Agr. Chem. Soc. Japan 32, 62, 1958)

In our previous experiments, it was shown that the distribution of proteins in the leafy vegetables undergo changes by the methods of drying, and also assumed that the decrease of nutritive value of leafy proteins is caused by these changes. In the present paper we studied on the digestibilities of various proteins in the green leaf alone of Soy bean by feeding rabbits. The results obtained are as follows:

The digestibiilties of the total crude protein, 0.3% alkaline 60% hot alcohol soluble protein and water soluble protein were 78%, 85.4% and 84.7%, respectively. On the other hand, the digestibility of 0.3% alkaline soluble protein was 68.7% and the latter was the lowest among them. On the reason of this fact, it is assumed that 0.3% alkaline soluble protein is not by itself digestible easily, but the decrease of digestibility of this protein is rather due to the larger amount of metabolic fecal nitrogen, because metabolic fecal nitrogen is soluble in 0.3% NaOH solution.

50. Studies on the water holding capacity of meats. Part 1. Water holding effects of several kinds of phosphates and commercial binding materials. Masao FUJIMAKI and Misako TSUDA (Journ. Utiliz. Agr. Products, 5, 62, 1958)

Water holding capacity of meats is increased by the addition of phosphates, but the effects are different for different kinds of meats, heating temperatures, storage time after addition of phosphates and quantities of mixed sodium chloride.

Results obtained did not agree with the theory that the effects of polymetaphosphates on meats are due to chelating reaction with calcium and magnesium ions in meats.

51. Studies on milk coagulating enzymes. Part 7. Comparison of α- and β-casein on the coagulability by milk coagulating enzymes. Kunio YAMAUCHI and Tomokichi TSUGO (Journ. Agr. Chem. Soc. Japan 31, 685, 1957)

Coagulation of unfractoinated, α- and β-casein by rennin, pepsin and papain was compared by measurements of the coagulating time in the presence of the same amounts of $CaCl_2$. At 35°C unfractionated casein was more coagulable than α-casein, while at 2°C only the α-casein was coagulated by rennin or pepsin. Papain differed from both in the respect that it coagulated unfractionated casein even at 2°C. β-casein was coagulated by $CaCl_2$ at 35°C though enzymes were not present, but at 2°C it was not coagulable by these enzymes.

More detailed study was performed on rennin coagulation. Into NaOH solutions of these caseins, rennin was added and heated to stop the action of the enzyme after a given time. The plotted curves of $CaCl_2$ concentration vs. coagulating temperature for these caseinate or paracaseinate solutions were obtained. From the comparison of these curves, it was concluded that the most marked change during the rennin coagulation of unfractionated casein will occur in α-casein and that difficulty in coagulation of it at lower temperature will be mainly attribute to β-vasein contained in it.

52. Moisture absorption of animal fibers in short time. Makoto KAMIYA (Jap. Journ. Zootech. Sci., **28** [6], 379-384, 1958)

Experiments on moisture absorption of dry original animal fibers were conducted and the following results were obtained,

1) Under definite conditions, dry original fibers showed different contents of maximum moisture absorption.

2) Changing phases of moisture absorption of these fibers are expressed in a common hygroscopic equation, that is,

$$y=B-\sqrt{\frac{K}{x-A}}$$

3) The hygroscopic constant (K) specific to each fibers was calculated from the equation by using the constants (A and B) related to time process (x) and moisture absorption contents (y).

4) The reliability of the equation was ascertained from the result that there was good agreement between the theoretical values calculated and the experimental values estimated.

5) The features of the hygroscopicity of animal fibers are considered to be related to their morphology.

53. Relation between moisture absorption of animal fibers and temperature or humidity. Makoto KAMIYA (Jap. Journ. Zootech. Sci., **29** [2], 103-108, 1958)

Hygroscopicities of dry original fibers of several animal species when influenced by fixed conditions (temperature or humidity) were examined, together, with their relations to three outside factors.

1) Maximum and minimum temperatures were correlated negatively with relative humidities, but such correlation was insignificant.

2) Animal fibers which had low values of minimum moisture absorption had low values of maximum moisture absorption, compared with other fibers. On the contrary, when the lowest hygroscopicity of some animal fibers was of the largest value, their highest hygroscopicity was also of the largest value.

3) Owing to the explanation of the relations of moisture absorption with the 3 surrounding factors, a linear regression equation was settled. Then regression coefficients, standard deviations of errors and significances of the regression were calculated.

4) Differences of regression coefficients expressed those of hygroscopic contents originated from the different structures of animal fibers.

Minimum temperatures had no relations with hygroscopicities, and maximum ones were correlated negatively with hygroscopicities, but such correlation was insignificant.

Relative humidities were correlated positively with hygroscopicities in wool, camel hairs and dog hairs. Apparently significant relations were present in rabbit and rat hairs. The correlation was very little in pig (Chinese and Barkshire) bristles.

5) From standard deviations of errors and the significance of the regression, it was concluded that no moisture absorption nor desorption of animal fibers reacted with variations of the three surrounding factors at the linear regression. It seems

to be necessary to take up regressions of higher order or consider some other reacting factors.

54. Studies on the morphology of animal fibers. Part 1. The morphology of fiber scales on the hair shaft. Makoto KAMIYA (Jap. Journ. Zootech. Sci., **29** [2], 106–116, 1958)

Microscopic examinations were carried out on the features in the morphology of animal fiber scales and the results obtained are summarized as follows.

1. No scale impressions of animal fibers were transformed by treatment with water, alcohol, and ether.
2. No difference in scale impression was observed between fibers of different breeds of the same animal species when the samples were of similar fineness.
3. The scale impressions of various animal fibers were divided into 6 classifications and were also related to the numbers of scales.
4. No relationship existed between the impressions or the numbers of scales and the animal species. The scale impressions were specific for each species.

55. Accumulation of alloisocitric acid by a *Penicillum* strain. Teruhiko BEPPU, Shigeo ABE and Kin-ichiro SAKAGUCHI (Journ. Gen. Appl. Microbiology, 3 [4], 225–232, 1957, Bull. Agr. Chem. Soc. Japan **32** [3], 207–211, 1958)

L_S-Alloisocitric acid, one of the diastereomers of isocitric acid contained in the Krebs' TCA cycle, was first found in nature as a new fungal product from glucose. This acid was isolated and identified as the γ-lactone from the culture medium of *Pen. purpurogenum var. rubrisclerotium* No. 1148. The paperchromatographic behavior of this acid agreed well with that of the synthetic alloisocitric acid. Final identification with the authentic specimen by the infrared spectrum and admixture test was achieved.

The acid was the only detectable acid accumulated by this fungus in the given condition and the yield of the acid by weight exceeded about 70% of the consumed glucose.

This was previously reported as a preliminary report in the Bull. Agr. Chem. Soc. Japan, **21**, No. 4, 1957, 263-264.

56. Studies on the metabolism of D-amino acid in microorganism. Part 4. On the nature of the purified D-glutamic-aspartic oxidase of *Aspergillus ustus*. Shôji MIZUSHIMA (Journ. Gen. and Appl. Microbiol. 3 [4], 233–239, 1957)

Further purification of the enzyme responsible for the oxidation of D-glutamic and D-aspartic acids was carried out and an enzyme of 90 per cent purity was obtained. This enzyme could oxidize D-glutamic and D-aspartic acids, but was incapable of oxidizing DL-α-aminoadipic and α-aminomalonic acids. The name D-glutamic-aspartic oxidase was designated for this enzyme instead of its previous name, D-monoaminodicarboxylic acid oxidase. The prosthetic group of the enzyme was proved to be FAD. No metallic component was detectable in the enzyme. Some studies concerning the mechanisms of the inhibitory action of KCN on the enzyme activity are also presented.

Part 5. Oxidation of D-glutamic acid by cell-free extracts of aerobacter strain A.

Kazuo IZAKI (Bull. Agr. Chem. Soc. Japan 22 [2], 78–84, 1958)

It is confirmed by a new enzymatic method for the determination of D-glutamic acid, that *Aerobacter aerogenes* strain A rapidly metabolizes D-glutamic acid, while it shows only a feeble metabolic activity towards L-glutamic acid when it is grown aerobically on a DL-glutamate-K_2HPO_4 medium at neutral pH value. A specific D-glutamic oxidase is demonstrated in the cell-free extracts of this strain. This enzyme seems to be different from D-glutamic-aspartic oxidase obtained from *Aspergillus ustus*, since the former has no activity towards D-aspartic acid.

Part 6. The occurrence of D-glutamic acid oxidizing enzymes in fungi. Shôji MIZUSHIMA (Journ. Gen. Appl. Microbiol. 4 [1], 12–20, 1958)

Most of the fungi tested in this study were found to be able to grow on D-glutamate. Several strains of them, belonging to *Aspergillus*, *Penicillium* or *Monascus*, could grow on D-glutamate as rapidly as on L-glutamate. In these fungi, two types of D-glutamate oxidizing enzyme were found; one was a specific D-glutamic oxidase and the other was a D-glutamic-aspartic oxidase. Most of the fungi tested were found to possess D-glutamic-aspartic oxidase, whereas, fungi belonging to *Aspergillus oryzae* possessed a specific D-glutamic oxidase. These D-glutamate oxidizing enzymes were remarkably formed when the fungi were grown on D-glutamate containing medium, whereas a little enzyme activity was observed in fungi grown on a peptone-glucose medium.

57. Cyanide insensitive terminal respiratory system in *Aerobacter cloacae*. Shôji MIZUSHIMA and Michiko NAKANO (Simposium on Enzyme Chem. Japan 283, 1958)

Aerobacter cloacae isolated from soil was found to show cyanide insensitive growth adaptively. The glucose oxidation by cyanide adapted cells was cyanide insensitive, whereas that by cyanide non-adapted cells was remarkably inhibited by cyanide. The oxidation of DPNH and succinate by cell-free extract prepared from both cyanide adapted and non-adapted cells was cyanide insensitive. This oxidase activity was mostly located in the subcellular particulate fraction. A cytochrome component which has maxima at 560 and 530 $m\mu$ was detected in the particulate fraction and was reduced by the addition of DPNH, succinate and dithionite.

58. Germination promotant for plant seeds, produced by microorganisms. Part 2. Germination promotant for rice plant seeds, produced by *Streptomyces* sp. S-580. Culture condition and extraction of the active crude syrup (A.C.S.). Yoshihisa KOAZE (Bull. Agr. Chem. Soc. Japan 22 [2], 91–97, 1958)

The culture filtrate of the *Streptomyces* sp. S-580 showed strong activity in promoting the germination of rice plant seeds. The culture conditions of the strain and fractionation of the active principles from the culture filtrate were investigated. The active crude syrup (A.C.S.) obtained is effective at the dilution of $10^{-6}\sim10^{-8}$ for the germination of rice plant seeds, and $10^{-5}\sim10^{-7}$ for barley and oat seeds.

Part 3. Germination promotant for rice plant seeds, produced by *Streptomyces* sp. S-580. Isolation and structure of an active neutral crystal (Factor-E). Yoshihisa KOAZE (Bull. Agr. Chem. Soc. Japan 22 [2], 98–103, 1958)

A neutral crystalline substance (Factor-E), which is effective for the germination of rice plant seeds at the dilution of $10^{-5} \sim 10^{-7}$, was isolated from the active crude syrup extracted from the culture filtrate of *Streptomyces* sp. S-580. And Factor-E has been proved by hydrolysis and synthesis to be L-proly-L-valine anhydride (diketopiperazine), which has been proved to be the new diketopiperazine.

Part 4. The germination promotant for rice plant Seeds, produced by *Streptomyces* sp. S-580. Isolation and structure of another active crystal (Factor-D). Yoshihisa KOAZE (Bull. Agr. Chem. Soc. Japan **22** [4], 283-248, 1958)

A crystalline substance (Factor-D), by which the activity of Factor-E in promoting the germination of rice plant seeds could be increased synergistically, was isolated from the active crude syrup from which Factor-E was also isolated. This Factor-D has been proved by synthesis to be 1-methyl-2, 4-imidazoledione (1-methylhydantoin). This substance has not been isolated from culture filtrates of any microorganisms.

III. Agricultural Economics

Abstract Nos. 59

59. On hog cycle. Keiji KAMIYA ("Economic Studies of Farm Management" in honour of Dr. Masao OTSUKI. Edited by S. Kashiwa. 1958)

The trend of Japanese agricultural development from the middle of the Meiji Era (1868-1912) to World War I was from production of starch to production of protein and vitamins. This tendency was reversed during and after World War II, but it was again resumed after 1951. It seems that there were some influences of governmental policy in making for this movement of agricultural production. Especially in rice production, the dominant force was political and administrative intention rather than farmer's voluntary economic intention.

If we could find an agricultural product which is free from any influence of governmental policy—in which producers, middlemen and consumers speak and act through their income—, such agricultural product would be a good material for economic analysis. We would then be able to apply true "iconic model" (in the sense used in the Operations Research) of producers having voluntary economic intention. The field of economic research of Japanese agriculture will be broadend through the use of such iconic models.

As models for economic analysis, we have analogue models and symbolic models beside iconic models. Hitherto we have been mainly concerned with symbolic models in economic analysis. We made big progress in using such models. Marginal productivity theory, of production function, output analysis and linear programming, are the examples of such models. Though statistical values can be substituted for variables representing capital, land and products, in such models, these statistical values themselves are derived from statistic-mathematical symbols like "population" and "samples", and statistics themselves are symbols like samples or average of samples.

The auther thinks that the gap between samples and models can be filled only by showing iconic models.

I will illustrate here the hog production as an example of iconic model of voluntary economic intention. Hog is one of the agricultural products which has not been objects of governmental policy. It has also an advantage that it is produced all over the country. Everywhere in the world, it is a common phenomenon that the cycle of pig production is independent of business cycle. In the course of business cycle, prices and output of most the of products move in the same direction. But in hog cycle, movement of price and output occurs alternatively. This is the reason why cob-web theorem is applicable to the analysis of the hog market.

In Japan, it is reported that there is a four year cycle in the price and the number of slaughtered hog. Correlation between both was-0.55. The regularity of the cycle was disturbed between 1940 and 1945, but we can see symptoms of repeat of a similar cycle after 1950.

We let p_t represent hog price at t th year and p_{t-2} hog price at $(t-2)$ th year. If we assume

$$p_t + p_{t-2} = \text{constant},$$

the cycle can be shown as a price movement with constant amplitude. Since 1955, the number of hog kept in farms increased very rapidly and it exceeded the former peak of 1953. It looks as if 1955 is a starting point of a new cycle. One of the reasons (on the demand side) for this increase seems to be the increase of national income which caused increased demand for processed foods. Production of hog carcasses was still roughly following its former four year cycle. If we deduct quantities of all processed meat (assuming it consisted only of pork) from production of hog carcasses and compare the difference with pork consumption per household of all cities (source of data: "Report of Household Consumption", Statistical Bureau of the Government), we can find that the coefficient of correlation between both is almost equal to unity for five years from 1952 to 1956. This fact seems to mean that consumption of hog carcasses can be distinctly divided into direct consumption in city households and demand from meat processing companies. Movement of the former is cyclical and that of the latter is a trend in character.

It must be noted that there is a tendency to increase also in the supply side (in agriculture). Most of the hog raising regions in Japan are in sweet potatoe producing areas. Decrease in demand for sweet potatoe as human food seems to have been made up by increase of demand as hog feed. Needless to say that this is one aspect of the trend of Japanese agricultural production (from starch production to protein production) which I stated at the beginning of this essay. Moreover, advancement in hog raising technique—progress of technology in producing ensilage and other feeds, application of chemical compounds (Aureomycin, Terramycin etc.)—has promoted hog production. Modernization of market facilities has also contributed to some extent.

Thus, it being clear that the rapid increase of hog population (stock of hog has been backed by favorable situations, both in production and consumption, we will pay attention to the movement of hog population. We assume that the rate of price rise, when hog price is rising, is in proportion to the difference between current stock and normal (given) stock. Let Q_t represent stock of hog at time t and write demand and

supply functions as

$$D=\alpha+ap$$
$$S=\beta+bp$$

where p is price of hog. Then.

$$\frac{dQ}{dt}=S-D, \quad Q=Q_0+\int_0^t(S-D)dt$$

Assume normal stock $\bar{Q}$ be given and let $\dot{p}$ represent rate of change in price.

$$\dot{p}=\lambda(\bar{Q}-Q)=\lambda\left\{\bar{Q}-Q_0-\int_0^t(S-D)dt\right\}, \quad \lambda>0$$

Differentiating this equation with t,

$$\ddot{p}=-\lambda\dot{Q}=-\lambda(S-D), \qquad \therefore\ \ddot{p}=-\lambda(b-a)p+\lambda(\alpha-\beta)$$

Equilibrium price p is determined by demand and supply functions.

$$\bar{p}=\frac{\alpha-\beta}{b-a}$$

Now let ρ represent deviation of p from equilibrium price $\bar{p}$ Then.

$$\ddot{\rho}=Q-\lambda(b-a)\rho$$

Solving this equation,

$$\rho=A\cos(\omega t-\varepsilon)$$
$$p=\bar{p}+A\cos(\omega t-\varepsilon)$$
$$\omega=\sqrt{\lambda(b-a)}$$

The cycle has constant amplitude and the period of the cycle is

$$\frac{2\pi}{\omega}=\frac{2\pi}{\sqrt{\lambda(b-a)}}$$

The larger λ and (b-a) are—the more sensitive the price in adjusting itself to the change in stock is—, the shorter the period of the cycle is. Period of four years is given when $\lambda=1.23$ and $-a=b=1$, or $\lambda=1$ and $-a=b=1.23$.

It is said that the cyclical movement of hog production was seen even after prices had been fixed. (Wageman: Narrenspiegel der Statistik p. 266). It might be a mistake to explain the hog cycle by income movement (J. Schumpeter: Business Cycle II, 430). To use too much analogy or too much symbolic elements in explanation may have a danger of missing the real nature of the problem. Now we will eliminate all exogeneous elements. We will stop to introduce income and price as variables in our model. Iconic stock of hog Q, quantity supplied S and quantity demanded D are enough for our analysis. The relation between these three variables is expressed as

$$\dot{Q}=S-D \quad \text{.......................................(1)}$$

There must be an ideal quantity of Q or f (S) which correspond to any value of S. The excess of f (S) over current Q will increase S after certain lag of time. D is assumed to be of given magnitude in the sense that it has no such relation with Q. Then

$$m\dot{S}=f(S)-Q \quad \text{..................................(2)}$$

We want to eliminate S from this equation and make it an equation with only one variable Q. namely an equation showing variation of the stock kept. Differentiating equation (1) with t,

$$\ddot{Q}=\dot{S}$$

Substituting $\ddot{Q}$ for $\dot{S}$ in equation (2),

$$m\ddot{Q}-f(S)+Q=0 \quad \text{..................................(3)}$$

We substitute equation (1) for S in above equation and obtain

$$m\ddot{Q}-f(\dot{Q}+D)+Q=0$$

If we let x represent

$$x=Q-f(D),$$

then

$$m\ddot{x}+F(\dot{x})+x=0 \quad \dots\dots\dots(4)$$

here

$$F(\dot{x})=f(D)-f(\dot{x}+D)$$

Equation (4) is called "Van Der Pol's Equation".
Movements exogeneous as well as endogenous follow the course indicated by this equation. When

$$F'(0)=-f'(D)<0 \quad \text{or} \quad f'(D)>0$$

equation (4) shows an "self-excited oscillation." So far as Q (stock) has a tendency to increase, the cyclical movement is repeated. If D is large enough to make f (D) negative and decrease stock (necessary for breeding), Q will decrease and we will see a remarkable decrease in numer of hog kept (1938-1947).

We have seen that a voluntary iconic model without trivial things as symbol or anlogue has stronger persuasive power. The auther thinks that the combination of Operations Research, which is linear in nature, with non-linear theories will be very effective in analysing a wide range of economic problems. Multiple linear theories are useful for analysing endogeneous movements. There are reasons for applying multiple-linear theories like Input-output Analysis or Linear Programming to the analysis of economy including agricultural products which are under strong political influences. But hog economy, of which voluntary intention is dominant, can be very well explained by applying non-linear theory.

But how has the energy which led to these voluntary intentions been prepared? This is one of the fundamental questions on which future prospects of Japanese agriculture depend.

IV. Agricultural Engineering

Abstract Nos. 60—70

60. A method of calculating potential evapotranspiration from pan evaporation data. Shigeyoshi SUZUKI and Hitoshi FUKUDA (Journ. Agr. Meteorology, Japan, **13** [3], 81-85, 1958)

In order to measure the actual value of potential-evapotraspiration (PE) (tank PE+rain—drained water) of upland rice and barley fields of volcanic ashy loamy soil, we used a tank, diameter 4 m, depth 50 cm, ground water level 45 cm from field surface, similar to that used by Thornthwaite.

The tank was surrounded by the same crops raised by ordinary method, where ground water level is morethan 15 m.

The amount of water evapotranspirated from the tank and also from a standard

pan used in Japan, its diameter 20 cm, hight 10 cm, were measured, and we obtained the following results.

a) The evapotransipration by Blaney and Criddles, Thornthwaite's methods were of less accuracy compared to that from the pan.

b) PE was found to be a function only of meteorological factors, regardless of the growth stages of crops.

c) PE was about 1.2 times the amount from the pan in each month of the growing season.

d) The actual PE is calculated with considerable accuracy by actual PE=tank PE in no-rain

$$\text{period} \times \frac{\text{mean daily amount of pan evaporation in each month}}{\text{mean daily amount of pan evaporation in no-rain period of the corresponding month}}$$

61. Under-drainage into ditches in soil overlying an impervious substratum. Hitoshi FUKUDA (Trans. Americ. Geophysical Union, 38 [5], 730–739, 1957)

Using conformal transformations, theoretical equations are dervied for the quantity of water, in water-saturated soil, seeping per unit time, from a plane water-table into equally spaced ditches with vertical walls. An approximate theory is also given for ditches which do not have vertical walls, Several of the theoretical equations are tested experimentally and are found to be in agrement with the experimental results.

62. Am example of a survey of water temperature of a reservoir near an intake, of Sudagai Dam. Shozo NOGUCHI (Study on the water temperature **1** [6], 1957)

Recently some complications have often arisen, in multi-purpose reservoirs, supplying water for both power and irrigation in this country, due to the low temperature of the water supplied for irrigation after being used for the generation of power. As such various intake works have been deviced for taking tepid water of surface layer.

Up to now, crest-overflow intake system is chiefly adopted as the intake system in high dams. In such types, there is a tendency for water of low temperature below the level of crest to be taken in, when the works are operated,

In this report, the author shows an example of his observations on the above conditions in the Sudagai Dam.

The Sudagai Dam is at the confluence of the main Tone river and its tributary Naramata river, which is 20 Km away from Minakami station, Gumma Prefecture, towards the upper reaches of Tone river and is 750 m above sea level.

It is a concrete gravity dam of the overflow type. 73 m in the height, 194.4 m in length, 210,000 Cu. m in volume, and has three tainter gates of 10.3 m in width at the overflow section of the spillway, and moreover, two intake towers having 8.1 m width of overflow part at the left side bank from the center of the dam. Summerized items of the reservoir are as follows: Catchment area 310 Sq. Km, Full water area 1.30 Sq. m, Maximum water depth 60 m, Designed Flood Discharge 1400 m/Sec. Storage capacity 28,500,000 Cu. m, water quantity for utilization 10-65 Cu. m/sec ordinary output 4,000 Kw, Maximum output 46,000 Kw, downward area for irrigation 400 ha.

For three days from 4th to 6th August 1957, the author observed the water temperature near an intake of the reservoir. As a result the following conclusions could

be made.

1. In the midsummer of August, the temperature distribution of surface water of the reservoir was 18.6-23.7°C in the daytime.

2. The temperature of surface water of the reservoir is strongly affected by atmospheric temeparture, and wind.

3. By the observation at every survey point from the intake, movement of water flow was remarkably recognized at the range of 1 m between the upper and the lower boundaries from a level of overflow crest under the conditions that a level of overflow crest from the surface water was 5.15~5.35 m and a quantity of water being taken in 20~50 Cu. m/Sec.

4. In overflow type intake works, the lager the width of crest, the more effective becomes the utilization of surface water, preventing cold water under the level of crest from being taken in when the intake works are operated.

5. During these observations, the temperature of the utilized water ranged from 14~15°C, about 4-8°C lower than that of surface water of the reservoir. In an intake where the flow exceeded 20 m^3/Sec, water above 16°C could not be obtained.

63. On the heat transfer and the liquid flow in salt-making. Part 2. On the viscosity of slurry. Jiro Sugi and others (Sci. Papers of Central Res. Inst. Japan Monopoly Corporation **100**, 89–95, 1958)

The viscosities of the slurry (This liquid contains solid particles of NaCl or $CaSO_4$) used in the salt industry, were measured. The observed values coincided with those calculated by using the formula of Mori and Ototake (1950):

$$\eta_r = 1 + \frac{K}{\frac{1}{\varphi_V} - \frac{1}{\varphi_{Vc}}}$$

where

η_r: relative viscosity K: constant

φ_V: solid fraction by volume φ_{Vc}: solid fraction at maximum concentration

64. Distribution of water falling down from the vertical flow concentrator "Shijoka". Jiro Sugi and Hiroo Inoue (Bull. Salt Sci. Japan, **12** [3], 137-147, 1958)

The distribution of the quantity of water falling down from the vertical flow concentrator "Shijoka" were studied. Within the range of experiments (40-240 L/Hr, the distribution of falling water has nearly no connection with discharge. As the number of sheets of bamboo-branches were increased, the point of maximum concentration in the volume distribution of falling water gradually moved forward. When three or four sheets of bamboo-branches (the angle of inclination 30° or 60°) were used, the maximum point arrived at the front edge of the sheet of bamboo-branches. If a piece of net (width of 10 cm) was stretched over the upper sheet of bamboo-branches under the water-supplying ditch. it has an excellent influence on the distribution. Moreover, on the basis of experimental results in case of a sheet of bamboo-branches, a method for the volume distribution of water was formulated.

65. Studies on the treatment of water and brine. Jiro Sugi and other (Sci. Papers of Central Res. Inst. Japan Monopoly Corporation, **100**, 61–81, 1958)

Part 1 On the treatment of sea watre and brine with slaked lime.

We studied the rate of removal of magnesium, purity and sedimentation velocity of magnesium hydroxide etc.; on treating sea water and brine. of various concentrations with slaked lime. The results showed, that the rate of removal of magnesium and purity of magnesium hydroxide, of sea water were good. With increase in brine concentration we observed that the rate of removal of magnesium and purity of magsium hydroxide decreased.

Part 2 On the concentration of sea water treated with slaked lime.

The results of experiments on the concentration of sea water treated with slaked lime, at the vat and model flow-down salt-field covered with vinyl sheets, show that calcium sulphate deposits earlier in the treated sea water (about 8° Be) than in the non-treated sea water.

Part 3 On the solubility of salt in brine treated with lime.

We investigated the character of saturated brine obtained by dissolving salt in brine treated with lime. We found that this treatment did not have any characteristic difference other than the fact, that the lime-treated brine dissolved a slightly larger quantity of salt as compared with non-treated brine.

Part 4 Removal of hydroxide. Removal of Ca^{2+} from brine treated with calcium hydroxide, for use in soda industry. Our experiments gave the following results.

(1) Removal of Ca^{2+} by Na_2CO_3 was effective. For example, the separated brine contained only 0.004% Ca^{2+}, in an equivalent reaction between Na_2CO_3 and brine.

(2) By the reaction between Na_2SO_4 and brine, 60-65% of Ca^{2+} was separated from original brine.

66. On the scale prevention by the method "addition of crystal seeds". Part 4. Jiro Sugi and others (Bull. Salt Sci. Japan, **12** [2], 96-113, 1958)

Our previous reports has dealt with the results of fundamental and practical experiments on scale-prevention by addition of crystal seeds. This new report is concerned with the relation between evaporation and properties of the seed itself. The following five experiments were carried out.

Exp. 1. The effect of suspended amount of seed.—The result showed that for practical purposes 20 g $CaSO_4$ crystals per liter of brine were adequate.

Exp. 2. The size of crystals.—The smaller crystals were more effective. Hence crushings of large crystals could also be used as seed.

Exp. 3. The influence of dihydratein anhydrite.—It was found that about 20% by weight of dihydrate in the anhydrite was permissible.

Exp. 4 The effect of temperature of the mother-liquor and water of crystalization.—The transition point was influenced by the seed. In the temperature range from 90-65°C, hemihydrate was relatively more effective. But after long running the evaporation was disturbed with scale formation.

Exp. 5. Effect of velocity of liquid circulated in heating-tube.—The quantity of adhered scale increased inversely as the velocity of liquid flow. Good data were obtained in this examination with a velocity of 0.25 m/sec.

67. On the synthesis of potassium selective exchange resin "polyhexyl". Part 1. Benzaldehyde as raw material. Jiro Sugi and others (Sci. Papers of Central Res. Inst

Japan Monopoly Corporation, **100**, 96–100, 1958)

A few reports were available on selective ion exchange resins. "Polyhexyl" which absorbed potassium ion selectively was first synthesized by A. Skobseid in Norway. The selectivity of "Polyhexyl" was superior to others. We made an attempt to apply this property in the salt industry, by synthesizing this resin, starting with benzaldehyde. We had to curtail this experiment due to economic reasons.

68. Studies on the distribution of threshed materials under the concave-sieve of a threshing machine. Part 2. Fusanobu SHOJI and Jitsuo YOSHIDA (Journ. Agr. Machin. Soc. Jap. **19** [3], 117–120, 1957)

The authors investigated on the distribution of threshed materials under the concave-sieve of a threshing machine with a self-feeder. The distribution of grains is shown at figure of the inclinded type from feed-side and its form is determined by feeding methods and condition of bundles. The bundles are mainly threshed at the first 1/3 part of threshing cylinder, and the 2/3 volume of threshed materials drops under the concave-sieve at that part. Wheats are threshed by higer effect more than rice-plants. Therefore, when we study on the problems of the winnowner under concave-sieve, it is necessary to consider on the distribution of threshed-materials, as above mentioned.

69. An analytical example on the threshing process of a threshing cylinder by high-speed camera. Fusanobu SHOJI and Fumihiko SANO (Journ. Agr. Machin. Soc. Jap. **19** [4], 167–170, 1958)

By means of high-speed kinematography taking about 900 photographs per second, the authors studied on the process of threshing a rice-ear with a threshing cylinder. The summary of conclusions reached from this experiment are as follows: The time required for threshing an ear is assumed to be about 1/100-2/100 second. There are two kinds of threshing function; one is the action that the teeth directly strike against an ear while touching, and the other is the action that the grains are shaken off on the occasion of swinging ears. Ears are forced to be oscillated by advancing teeth and threshed materials are fairly scattered on the side directions.

70. A Kinematographic study on the feeding-out function of paddy with feed-rolls of rice huller. Fusanobu SHOJI (Journ. Agr. Machin. Soc. Jap. **20** [1], 37–40, 1958)

The author studied on the functions of six feed rolls of the rice huller, by means of high-speed kinematography taking about a maximum of 1600 photographs per second. Revolution of rolls is given in high and low respectively. The relation between peripheral velocities of rolls and flowing-down velocities of paddy, the distribution percentages of paddy-mass flowing at 4 sections of 2½" width, the fluctuations of their discharge, and the percentage of directions of flowing-down paddy were clarified according to measurements of films.

Generally speaking, the functions of rag rolls are on the whole satisfactory, especially the roll with less rag supported at both ends is assumed to be better. The roll with toothed discs is not suited for a paddy feeder of rice-huller, because it is difficult to give the equal feeding and to arrange the longitudinal axis of paddy. Roll with deep flute is fitted to the external force feed of seeds.

V. Fisheries

Abstract Nos. 71—97

71. Productivity of plankton-feeders in the off-shore area of Japan. Yoshio HIYAMA and Fujio YASUDA (Rec. Oceanogr. Works in Japan 4 [2], N. Ser.: 193-199, 1958)

Roughly speaking, the area where the Japanese fishing activities are operating could be divided into three areas, according to the distance from the coast, namely: (1) Coastal area, (2) Off-shore area, and (3) Oceanic area. Though it is hard to define by the accurate measure of distance from the coast, the off-shore area, here the authors are going to deal with ,is an area beyond the coastal area and about 5 to 50 miles off from the coast. This area is generally operated by medium sized boats, of about 20 to 50 tons, furnished with about 50 to 100 H.P. engine. The length of fish cruise is often one day or night, and it is rarely a few days.

This area had been explored since thirty or forty years ago by the development of fishing boats of the modern type. Satisfactory historical statistics for the production and for the number of boats is available for the authors' reference.

The total production from these areas occupies about half of the total Japanese landing, fished by about 4,000 boats in recent years. Though a part of the catch is bottom livings, like plaice, turbot, crabs, shrimps and others, a much bigger part of the catch is either surface or middle-layer livings, such as sardine, anchovy, herring, mackerel, skipper, squids and other plankton-feeders and carnivorous fishes such as yellow-tail, cod, skip-jack, tuna, salmon and others.

In the products from the off-shore area, which arises to one and a half million metric tons per year in some period, plankton-feeders occupy about 90% and are about 10 times of the carnivorous fish, in amount.

The historical review on the statistics of the amount of landing of these plankton-feeders from the off-shore area shows that the total landing of these species altogether is nearly the same in both the pre-war time and post-war time, though that for each species changed remarkably and the abundance for them also changed. This is a peculiar circumstance in the world, and the authors here have tried to analyse how and why it is so.

72. Morphometric comparison of the yellowfin tuna taken from the Equatorial Pacific. Kenji KUROGANE and Yoshio HIYAMA (Bull. Jap. Soc. Sci. Fish. 23, 388-393, 1957)

Of the sixteen morphological characters of the yellowfin tuna, *Neothunnus macropterus*, distributed in the Equatorial Pacific ranging from 130°W to 130°E, biometrical comparison was made, basing on the morphometric data of 263 specimens, so as to know whether the fish that the Japanese tuna boats are catching belong to one and the same population or not. The following results were obtained from this study.

1) The specimens of yellowfin tuna taken from the same area in dicerent years have considerably similar morphometrical characteristics, excepting for a few rharactess that show variation.

2) There appears to be a much greater degree of separation between 170°W

and 150°E and between 160°W and 130°W, than between 150°E and 130°E and between 170°W and 160°W.

3) The specimens of yellowfin tuna from the area of 160°W have shorter head, more anteriorly positioned ventral and pectoral fin than those from the eastward and westward regions. But for eye diameter, second dorsal and anal length, the measurement is the greatest in samples from the Western Pacific and the least in the Eastern Pacific samples.

4) It is morphometrically probable that there would be at least three independent populations with some mixing between each other.

73. Morphometric characteristics of the albacore, *Germo germo*, from the Northwest Pacific. Kenji KUROGANE and Yoshio HIYAMA (Rec. Oceanogr. Works in Japan 4 [1], N. Ser.: 67–75, 1957)

In the Northwest Pacific two kinds of fishery for albacore, the pole-and-line and the long line, have been in operation. The pole-and-line is carried for relatively small albacore in early summer near the coast, and the long line fishery for relatively large fish during winter in the off-shore waters. Of the seventeen morphological characters of these catches, the biometrical analysis was made, basing on the morphometric data of 288 specimens, before comparison of other groups caught from various localities in the Indo-Pacific area are started. From this study, we could determine, what regression lines fit the relative growth of each character; what character has a great variability within the same population; and whether or not the gear and the body size affect the morphometrical characteristics of the albacore.

The rate of growth of the pectoral fin is less than that of the body length over the range of sizes examined. The relationship between pectoral fin length (y) and body length (x) may be well shown by an equation of the type $y=a+b \log x$. There seem to be slightly an inflection at about 70 cm. body length size in the relative growth of several characters, such as head length, the fin insertions and fin lengths etc.. Accordingly, when we discuss about the specimens divided into sub-samples at about 70 cm. body length, these characters fit well in a straight line graph.

It is noted that the morphometrical characteristics of the albacore under 70 cm. body size differ in the regression coefficients with those over 75 cm., taken by the pole-and-line fishery, while in the comparison of the same size samples and of the albacore over 75 cm. taken by two different gears, the difference is not recognized in the regression coefficients but in the adjusted means. The difference of regression coefficients seems to be affected by the size composition of samples. In the comparison of the same sized albacore, the variability is rather great in the relative growth of body depth, distance anal to caudal and maxillary length. In the comparison of the different two gears albacore, the specimens of albacore by the pole-and-line have more anteriorly positioned anal fin, higher body depth and lower spread caudal than the long line albacore. Though we could not find any precise factors to produce such heterogeneity, it is necessary to take such results into consideration, when we judge the differences between regions.

74. Morphometric comparison of the albacore from the Northwest, the Equatorial and the Southwest Pacific. Kenji KUROGANE and Yoshio HIYAMA (Rec. Oceanogr. Works

in Japan 4 [2], N. Ser.: 200–209, 1958)

The albacore is distributed abundantly in the Northwest and the Southwest Pacific all the year round. Occasionally, the fish is also caught in plenty by the Japanese long line tuna boats in the Equatorial Pacific. The problem of whether or not these albacore are composed of a number of independent units, was analysed in this study from the standpoint of morphometry. In addition to the Northwest Pacific samples that were reported in our previous paper, we collected one sample from the Southwest Pacific and four samples from the Equatorial Pacific ranging from 150°E to 160°W. The following results were obtained.

1) In the comparison of the four samples from the Equatorial Pacific, body depth, maxillary length and diameter of iris show great variability. However, in many body characters great differences are not recognized and we may consider that the four samples from the Equatorial Pacific belong to a simple population.

2) The specimens of albacore from the Equatorial Pacific (under the South Equatorial Current) are similar morphometrically with that of the Southwest Pacific. We may deduce that the albacore of these two areas belong to a common population and the latter migrate northwards in the winter season.

3) The specimens of albacore from the Northwest Pacific have shorter head and maxillary, more anteriorly positioned fin insertions, lower first dorsal spine and spread caudal, longer body trunk and caudal regions than those from the Equatorial and the Southwest Pacific areas. Basing on these results, we may consider that the Northwest Pacific albacore form a distinct unit from the Equatorial and the Southwest Pacific albacore.

75. Experiments to see the effect of colour and its depth on the driving net by the centralizing method. Takaya Kusaka (Bull. Jap. Soc. Sci. Fish. **23**, 766–769, 1958)

This is a continuation of the earlier work which was carried out by a model net made of coloured vinyle cord to know the effect of it on the drive of fish. 10 Goldfish *Carassius auratus* of an average total length of 3.5 cm. were liberated in a wooden tank of 50 cm. long, 26 cm. wide and 10 cm. of water depth. A pair of model nets were prepared with wooden frames holding a stretched netting of small mesh, excepting the middle portion which was left open to insert three glass tubes in it. For observing the effect of colours, red, blue and black inks to fill each glass tube was used, and for depth of colour, these inks were diluted in 10-1,000 times. The centralizing method was repeated 40 times using all these 11 conditions and in all combinations of two conditions each. The sum of the total values are given and to

Table. Orders on the driving effects and visible distance in the water for the frames

Kind of frame	Frame without tube	Dark red	Middle red	Light red	Dark tube	Middle tube	Light tube	Dark black	Middle black	Fresh watet	Hollow tube
Order of driving effects	(11)	(1)	(3)	(4)	(2)	(5)	(9)	(6)	(7)	(10)	(8)
Order of visible distances	—	(3)	(6)	(9)	(1)	(2)	(7)	(5)	(4)	(10)	(8)

them was applied the chi-square test of statistics. Also the visible distance of the glass tubes in the water are given. The values compared are shown in the table.

Since the driving effect is influenced by the difference of the kinds of colours, the author recognized superiority and inferiority of the effect according the depth of colour in the same. The visible distance of those glass tubes in the water coincided with the order of depth of colour, nevertheless the colour, more visible in water is not always excellent on the driving effect. As coinciding with the result of the former report, the red ink of non-dilution is most excellent on driving effect. To sum up these conception, the author thinks that the distinctness of kind and depth of colour is excellent for producing driving effect.

76. Movements of the fish in response to sound stimuli with reference to sound-intensity. Yasuo SUYEHIRO, Shizuo YOSHINO, Yoshikazu TSUKAMOTO and Makoto SAITO (Jap. Journ. Ichthyol. **6**, 136–140, 1957)

The authors performed the experiments to ascertain the effect of the sound stimuli to the behaviour of fish, by varying the sound-intensity.

The behaviour of fish in response to the sound stimuli were observed by using fire-work under the water and by striking the bottom of the cask as the source of sound. It is now made clear that the critical sound-intensity to affect the behaviour of fish varies from one species to the next, and mackerel (*Scomber japonicus*) and horse mackerel (*Trachurus japonicus*) are rather sensitive fish, however conger eels except *Rhyncocymba nystromi nystromi* are less sensitive.

77. Studies on the adrenal glands of teleosts. Part 2. On the adrenal tissues in fifteen species of fishes. Mikio OGURI and Takashi HIBIYA (Bull. Jap. Soc. Sci. Fish. **23**, 144–149, 1957)

Arrangement and nature of the acidophile cells and the chromaffin cells in head kidneys of fifteen species of teleosts are described from the histological point of view, and also some details about the corpuscles of STANNIUS of them are added.

Surrounding the cardinal veins and their branches in the head kidneys, the acidophile cells are found as densely packed cell groups or as irregular cell-cords. On the other hand, the chromaffin cells appear in the walls of cardinal veins and their branches, sometimes scattering in the acidophile cell groups.

The acidophile cells are stained deeply with eosin and contain relatively large round or oval nucleus, in which a well defined nucleolus and a small number of chromatin are found. The chromaffin cells are stained faintly with eosin. The nuclei of chromaffin cells are oval or irregular in shape, and larger than those of the acidophile cells.

Concerning the arrangement of the acidophile cells and the chromaffin cells, the following six types are distinguished.

Type 1: In *Carassius auratus, Cyprinus carpio, Misgurnus anguillicaudatus, Channa argus* and *Plotosus anguillaris* the groups of acidophile cells lie around the cardinal veins and their branches and the chromaffin cells are scattered as single cells or cell groups in those acidophile cells.

Type 2: In *Parasilurus asotus, Niphon spinosus, Pterois lunulata, Lepidotrigla microptera* and *Hoplobrotula armata* the chromaffin cells appear in the walls of the

cardinal veins and their branches, while the groups of acidophile cells surround these veins.

Type 3: In *Salmo gairdnerii irideus* the acidophile cells are never in contact with the chromaffin cells but are situated separately around the small veins in the lymphoid tissue of the head kidney. On the other hand, the chromaffin cells are found in the wall of the cardinal vein.

Type 4: In *Prionurus microlepidotus* and *Hexagrammos otakii* the groups of acidophile cells surround the cardinal veins and the chromaffin cells are distributed independently around the small veins. Furthermore, the small groups of chromaffin cells are scattered among those of the acidophile cells.

Type 5: In *Paralichthys olivaceus* the cords of the acidophile cells and those of the chromaffin cells are intermingled as in the adrenal glands of birds.

Type 6: In *Anguilla japonica* both of the acidophile cells and the chromaffin cells are not found in the lymphoid head kidney, but they are confined within the wall of the sinous vein near the head kidney.

The corpuscles of STANNIUS do not exhibit histologically any wide variation throughout the different species of teleosts. The cells of these corpuscles contain many siderophile granules, which are stained well with iron-hematoxylin, azocarmine or eosin. Moreover, these granules are PAS-positive, but contain no lipoid droplets.

78. A study of the lateral-line system in fish. Part 1. Quantitative analysis of fiber calibers in the lateral-line nerves of fishes. Yoshikazu TSUKAMOTO and Shizuo YOSHINO (Jap. Journ. Ichthyol. **6**, 59–64, 1957)

The quantitative analysis of fiber calibers in the lateral-line nerve of fish was done on 81 species of teleosts by using unfixed and living fibers. This nerve consisted of many kind of fibers which differed in diameter, however the individual variation of fiber distribution was not found in the same species. Therefore, we were able to compare the nature of the fiber in many species of fish by measuring fiber calibers.

Looking over all the fishes studied in the present experiment, the following three types might be distinguished in their fiber distribution.

1) The nerve consisted, for the most part, of relatively thin fibers, and most of them had a diameter of 4μ to 6μ, a smaller proportion, 6μ - 10μ, and a few 3μ. No fiber with diameter exceeding 10μ was observed.

2) The fiber varied from 3μ to 15μ in diameter, and the greater part of them had diameters of 6μ to 10μ.

3) The fibers were thicker than the other two types mentioned above. The mean value in diameter was over 8μ in all species, and the range of deviation in caliber was so wider than the types 1 and 2.

Thus, it was noticeable that there were these three types on all fishes, both in marine and in fresh water, therefore we could divide these 81 species into three different groups.

Part 2. On the relation between the function of the lateral-line organ and the habits of fish. Yoshikazu TSUKAMOTO and Shizuo YOSHINO (Jap. Journ. Ichthyol. **6**, 131–135, 1957)

In this paper the relationship between the function of the lateral-line organ

and the habits of fish from the ecological stand point of view is discussed.

We could divide 81 species of teleosts into the following three groups according to their fiber distribution.

Group I. Some fresh water fishes, such as crussian and killifish etc., live in the pond or in the calm river. Some marine fishes, such as rocky fish, goby and sea catfish etc., do not show active movement, but stay in under-water-cave or on the side of the rock or in the tide pool. They are relatively inactive and sluggish fishes, and they swim rather solitarily and seldom migrate or roar over wide areas.

Group II. These are the good swimming, schooling, and migratory fish. Most of them, such as anchovy, mackerel and yellow tail, swim in groups attractively. They have stronger swimming force, and they are the more active and excellent migratory fish, which rapidly schools. Mostly, they are adapted for rapid movement by being "stream-lined", either fusiform or laterally compressed. They are known to swim near the surface or in the middle layers of the water. There are also found some nocturnal fish in this group.

Group III. There are found in this group migratory fish, nocturnal fish, bottom feeder and flyingfish. Salmon, pike, etc., are good swimming and migratory fish. Some fishes in this group are known to remain quietly on the bottom. These fishes are adapted to the life at the bottom by having the body-form angular or depressed, eg. the flatfish and sole. And then eel-like fishes belong to this group, and they are almost nocturnal fish. In addition to these fishes, we can nd the strange habitant, which fly often over the water, eg. the flying fish.

It may be concluded that the function of the nerve fibers in size, ranging from 6μ to 10μ are very significant in the life of fish in general. We could determine the correlation between the function of this organ and the habits of fish. Results of our studies lend support to the view that the habit of the fish belonging to any one of these three groups may depend upon the function of each individual nerve.

79. Cultivation and preservation methods of marine protozoa. Reijiro HIRANO and Yasuo OHSHIMA (Bull. Jap. Soc. Fish. **23**, 420–422, 1957)

Recently, considerable attention has been paid to free-living marine protozoa as food for the rearing of marine animal larvae. However, even after a long period of experimentation, many different problems on the collection, isolation and cultivation of these free-living marine protozoa still remain to be solved. The present paper reports the preliminary studies made on the cultivation and simple preservation methods of marine protozoa. The method aims to make it possible to supply food organisms whenever it is needed.

The writers succeeded in the cultivation of five species of free-living marine protoza (four species of ciliata and one of flagellata) isolated from marine alage, detritus and sea water, collected from the tidal zone around Aburatsubo Bay, near Misaki in Kanagawa Prefecture. A culture medium of 200 p. p m. of glucose added to Allen-Miquel-Nelson Solution seemed to be the most suitable one for the culture of marine protozoa. However, for the cultivation of flagellata, the medium when algae-extract or dried activated sewage-sludge-extract added showed better results.

The writers also succeeded in the encystment of cultivated protozoa by their

new filteration method of which two factors, desiccation and crowding, may have contributed to the cyst formation of their cultivated organisms. The filteration method may be generally siutable for the encystment of marine protozoa. Furthermore, this method is not only useful for the encystment of the protozoa, but also useful for the concentratin and desiccation of the organisms.

In our laboratory, five cultivated protozoa, preserved in a special form at room temperature for 10 months, have been observed to show normal excystments. The cysts were preserved in special "cyst powder" form, i. e. a mixture of cyst and a sufficient quantity of filter substances containing alumina and gypsum, by the method mentioned above.

80. On the culture and ecdysis of the Phyllosoma in the spiny lobster, *Panulirus japonicus*. Tadashi NONAKA, Yasuo OHSHIMA and Reijiro HIRANO (The Aquiculture 5 [3], 13–15, 1958)

With a view to learn the metamorophosis and growth of the Phyllosoma, the larval stages of the spiny lobster, *Panulirus japonicus*, the breeding of the first larval stage was attempted by many investigators, but their efforts ended in failure. In the summer of 1958 the breeding experiment was carried out by the authors at Misaki Marine Biological Laboratory of Tokyo University and it was proved that the larva could be fed with newly hatched brine-shrimp and the first ecdysis could be observed. This paper deals with the results of breeding experiments carried out after that. The results obtained are summerized as follows:

The Phyllosoma, hatched on August 30th, was reared in glass finger-bowls (15 cm in dia. and 10 cm in depth) and glass vessels ($15\times10\times20$ cm), with the result that 24 days rearing was successful. During this rearing period water was renewed twice and temperature kept between 25-27°C.

The larva fed with brine-shrimp performed the first ecdysis on the 10th. of September, and the second on September 23rd. The second stage grew to 1.70 mm in body length and the third to 2.03 mm. On the other hand the ones which abstained from food, all died within a week. Brine shrimp was effective as food at this larval stage, even though it may not be the best of foods in this instance.

Each successive larval stage was identified by the following points: (a) In the second stage, the segmentation of the eye-stalk appeared, (b) In the 3rd. stage the exopodite of the 3rd. pereiopod segmented. The appearance of the 4th. pereiopod can be expected on and after the 4th. stage and the segmentation of the first antenna is only after the more advanced stages.

In the light of the morphological character of the third larval stage it seems possible to assign the Phyllosoma, Form E, of genus *Panulirus* described by Y. OSHIMA in 1942, to the *Panulirus japonicus*.

81. Effect of suspending particles on the pumping and feeding of marine bivalves, especially of Japanese little-neck clam. Kenji CHIBA and Yasuo OHSHIMA (Bull. Jap. Soc. Sci. Fish. **23**, 348–354, 1957)

Marine bivalves usually obtain their food by means of filtering the plankton and other suspending particles with their gills. So the quality and quantity of such materials should have some effect upon their life and existence. In this study some ex-

periments were carried out to investigate its effect on the rate of pumping and feeding of bivalves under comparatively high concentrations of suspending particles in sea water, using the following marine bivalves as materials: *Tapes semidecussata* (Japanese little-neck clam), *Meretrix meretrix lusoria* (Hamaguri), *Ostrea gigas* (oyster) and *Mytilus edulis* (sea-mussel). The results are summerized as follows.

1) The bivalves were placed in sea water containing 10-20 mg/litr. of such organic or inorganic particles as *Scenedesmus sp.*, *Thalassiosira sp.*, diatomaceous earth, kaolin, bentonite, talc, shale, charcoal powder and soluble starch. Their sizes range from 3 to 60μ. This concentration of particles was considered rather high in nautral condition, but the bivalves never stopped the pumping, although the pseudo-feces was often excreted, and these particles were clearly swallowed, irrespective of their quality, whether they are organic or inorganic.

2) The rate of pumping was computed by the formula of Jφrgensen: in $P_t/P_0 = -R/L\ t$ (where P_0 and P_t are the concentration of particles at the beginning of experiment, and, after a time t, R is the rate of pumping, L is the volume of sea water used.). The concentration of particles was measured by an electrophotometer, but the concentration under 30 mg/litr. was difficult to measure. The rate of feeding was measured by means of weighing the dried feces excreted by the bivalves an hour after they had been placed in the sea water which contained suspended particles, and at the same time the pseudo-feces, excreted by them while they were in the turbid sea water was weighed. Bentonite was chiefly used as the material of suspending particles.

3) In each species of bivlales it was observed that the rate of pumping was scarcely reduced by the influence of high concentration of bentonite, up to 500-1000 mg/litr.

4) *In Tapes semidecussta* the amount of feeding increased with the growing concentration of bentonite up to the time when the concentration grew higher than 10 mg/litr., and it was at this stage that pseudo-feces was discharged, and in higher concentrations the pseudo-feces insreased further, whereas the true feses did not increase. Nearly the same tendency of feding was observed in *Meretrix meretrix*.

5) It was found in *Ostrea gigas* and *Mytilus edulis* that the amount of feeding increased with the growing concentration of bentonite, but the rate of its increment grew smaller.

82. The present status of *Mugil* culture in Aichi Prefecture. Nakaroku NAKAMURA (Aquiculture 5 [2], 41–47, 1957)

In many creeks and brackish ponds in the sea-side region of Ama gun, Aichi Prefecture, the mullet (*Mugil cephalus*) is cultured extensively and its yearly total yield amounts to 120-200 tons. This amount of mullet yield is never small in comparison to the area of water under culture. The culturing method such as absolutely non-feeding method is attractive.

In the case of grouping 35 pisci-culturists who form the association, into 4 classes according to the yearly income, 3 belong to A class (more than 5 million yen), 5 to B class (ca. 2 million yen), 6 to C class (ca. 1 million yen) and 21 to D class (ca. 0.5 million yen). While in the pisci-culturing waters, the mullet, carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), eel (*Anguilla japonica*), sea-bass (*Lateolabrax*

japonicus) and sometime the black porgy (*Mylio macrocephalus*) etc. are cultured together, the mullet (ca. 67.4% in the amount of yield, or ca. 72.7% in the price sold) is the most important fish.

The most urgent problem in the present status of the pisci-culture now under consideration is the lowness of survival rate of the mullet fries, which is mainly due to the high mortality of them in the water of low chlorinity content. Because, in recent years, the mullet fries that come along to the estuary and the sea-side in early spring, are very scarce in this district and its adjacent waters, the difficulty of releasing sufficient amount of fries is now becoming a bottleneck to this enterprise.

One method considered to dissolute this bottle-neck is to prepare some special ponds of which water contains sufficient chlorinity to the safety of the mullet fries, culturing them in a short period while the fries grow up to have more resistance to low chlorinity.

83. Effect of water tempetature on the growth of sea weed. Part 1. Seibin ARASAKI (Aquiculture 5, 60–65, 1958)

The temperature of coastal water is, of course, an important factor influencing on the growth or phenology, and distribution of the sea weeds. When we think about the relationship between the temperature and the phenology of a plant at any place, or consider on the causative factors of different growing phenomena of the same species at different places, it is more pertinent in the explanation of the problem, to deal with the change of monthly mean value of water temperature than to do with the yearly mean value. The difference in the phenolgy of the *Monostroma, Porphyra,* and a marine phanerogam *Zostera nana* at several different places along the Japanese shore, especially those in Tokyo Bay and Ise-Mikawa Bay, are discussed dealing especially with the change of monthly mean value of water temperature at each place. A considerable difference occurs usually in the summer period, July and August, and that is to say, it correlates to the temperature, whether it surpasses over 25°C or not. From these facts, the author gave an important meaning in the field of algal ecology to "the frequency and the sum of deviated figures over 25°C in the monthly mean value of the temperature", and prepared for it the term "Indicator of the heat".

84. On the algicide Delrad 70. Seibin ARASAKI and Koji NOZAWA (Bull. Jap. Soc. Sci. Fish. **23**, 599–603, 1958)

There are many chemicals for killing or eliminating the weeds and fungi, but very few of them have proved successful in controlling submerged aquatic plants or algae. It is said that Delrad, manufactured at Herculus Powder Co. in U.S.A. is specially effective against the algae. In the present investigation, the authors dealt with the action of Delrad 70 to some fresh water algae such as *Chlorella, Scenedesmus, Oedogonium, Microcystis,* and *Oscillatoria,* and also to a water plant *Hydrilla.* The efficiency of the agent was estimated by the photosynthetic activity measured through mannometric method, or by macroscopic observations on the discoloration and sedimentation of algal cells.

The results obtained were as follows: 1) The value of active concentration of Delrad 70 varied from 0.1 to 1.0 ppm. depending on the kind of algae or their population density. In dense culture of algae, the efficiency decreased probably due to

the adsorping activity of the agent to the algal cells. The agent seemed to be harmful to gold-fish in almost the same concentration as to the algae. 2) The degree of adsorption, or the decrease of efficiency of Delrad 70 was great in water with mucous excreta of gold-fish or with dense population of *Scenedesmus*, while not so great in water with kaoline suspension.

85. On the pathogenetic water mold. Part 1. Seibin ARASAKI, Koji NOZAWA and Makoto MIYAKE (Bull. Jap. Soc. Sci. Fish. **23**, 534–538, 1958)

In the aquiculture ponds or in the aquarium of tropical fishes, the fungi-disease occurs frequently, and brings about severe damages to fishes. The experiments and observations on the life-cycle and pH suitable for growth of pathogenetic water mold, *Saprolengia*, were carried out. Many materials isolated from diseased trouts, tropical fishes, and eels, and diseased eggs of trout and *Hypomesus*, etc. which were collected from several places in middle Japan were used for the investigation. The results were summarized as follows:

1) Almost all the fungi were identical with *Saprolegnia parasitica* COKER. Some of the rest semed to be other strains belonging to the same species, but could not be correctly identified. A few believed to be of the other strain that has a rather stronger resisting ability against malachite green. 2) In simple pepton medium, the mycellium continued only to grow, whereas in distilated water it formed zoosporangia. Gemma were formed in low temperature condition (below 15°C) or after a long culture period. Considering that the gemma formed frequently numerous zoospores in themselves just as zoosporangia did, it seemed that there might be essentially no difference in the nature between zoosporangium and gemma. 3) The growth rate of the mycellium measured in volume was shown as a sigmoid curve that has a rather longer straight lined part. 4) The same strain had almost the same top-point in optimal pH value, near the neutral point, for growth. But in some cases, they seemed to manifest an after-effect of their native ponds. 5) The pH value of the culture-medium gradually approached to the optimal one in the course of experiment. Correlation between the growth of mycellia and pH in agar-plate culture was quite different from those in solution culture.

Part 2. (Bull. Jap. Soc. Sci. Fish. **23**, 593–598, 1958)

In order to know the effective chemicals for controlling the growth of *Saprolegnia*, many chemicals such as agricultural fungicides, medical agents, and pigments were tested. Among these usupulun (an organic Hg-salt), crystal violet and marachite green were most effective. In this investigation we dealt especially with the malachite green (MG).

The capacity of *S. parasitica* resisting to MG was variable according to the life-time: i.e. the growth of hyphae was completely restrained in pepton-solution with MG of 0.02 ppm, while the formation of zoosporangia and gemma, or the development of resting spores was checked with 0.2 ppm. The correlation diagram between the concentration of MG and the time needed to kill the hyphae or the sporangia was shown as a straight line when both factors were expressed in logarithum. As the infection of the disease is brought principally by zoospores, in practical application the dosage should be effective to them. It was suggested that it might be enough to treat the

trout egges for about 20minutes in MG of 5 ppm, and as to the eels it might be necessary to use a more concentrated solution than 0.2 ppm.

86. Studies on the protoplasm of algae. Part 3. The correlation between salinity of sea water and toxicity of ammonium sulfate. Koji NOZAWA and Yuriko NOZAWA (Bull. Jap. Soc. Sci. Fish. 23, 423–426, 1957)

In order to clarify the influence of the salinity of sea water and the toxicity of ammonium sulfate on the laver (*Porphyra tenera*), the present investigation was carried out through the following designs: 1) the media covering from 0 to 34‰ Cl were prepared in 2‰ Cl intervals from the nautral sea water, or from the artificial sea water of Levring's solution, and then the ammonium sulfate was added to these in 0.001, 0.003, and 0.1 Mol. respectively. 2) the laver fronds were immersed for one or two days in the media of varied chlorinity and varied concentration of ammonium sulfate. 3) the rate of efficiency was estimated by microscopic observation, and from the deformation of the cells it was classified into five degrees.

The results are summarized as follows:

a) As to the chlorinity, the cells a deadly attacked in lower chlorinity of 0∼4‰ Cl, considerably injured in 6∼10‰ Cl, and over than 22‰ Cl, they are normal, and sound in 12∼20‰ Cl.

b) As to the ammonium sulfate, the cells are considerably injured even in 0.001 Mol. The effects are always greater in dilute sea water below 10‰ Cl than the rest, or the normal (12∼20‰ Cl) and the concentrated (22∼34‰ Cl) ones.

c) It is suggested that hte adaptability of the laver-cells to the diluted or concentrated sea water deviated far from the normal ones seems not to be so great as has been thought.

87. Studies on the disease of the *Conchocelis*-phase of *Porphyra* (Preliminary note). Yuriko NOZAWA and Koji NOZAWA (Bull. Jap. Soc. Sci. Fish. **23**, 427–429, 1957)

In mass-culture of the *Conchocelis*-phase of *Porphyra tenera* which penetrates in the oyster shell, there is often observed a disease showing many orange-yellow spots on the shell surface. Sometimes, this disease causes fatal damages to the *Conchocelis*.

After the fixation by Perny's solution, and staining by haematoxylin, methylene blue or methyl violet, the authors found many individuals of rod shaped bacteria within the affected part of *Conchocelis*-filament. The present bacteria are found only in the diseased part, but not in the healthy part of the same *Conchocelis*-filament. Moreover, the authors were able to isolate about nine kinds of bacteria on agar-plate from a piece of oyster-shell with diseased *Conchocelis*. Among these, only one strain can infest into the *Conchocelis*.

It still remains uncertain whether the bacteria isolated from the *Conchocelis* acts as an essential cause or secondary one for the present disease.

88. On the culture of "WAKAME" *Undaria pinnatifida*. Yûnosuke SAITO (Aquiculture 5 [3], 16–20, 1958)

Although the culture of "Wakame" (*Undaria pinnatifida Sur.*) is going well industrially in the northern waters of Japan, it has never succeeded in the middle and southern area. With the view of clarifying this reason, some experiments were

carried out in Ise Bay through 1957.

Seedlings of "Wakame" attached to the oyster shells were cultured indoors throughout the warm season, and were submerged in various depths in the sea on Aug. 1, Sept. 14, Oct. 25, Nov. 21, and Dec. 14 respectively. Judging from the results obtained, it has been cleared that the number of germlings and growth of fronds vary with depth under water, at each time of submerging seedlings, and also this phenomenon seems to have bearings upon water temperature and illumination in sea.

Consequently the depth where the seedlings are submerged and the time of beginning of culture must be considered of having some connection with the above mentioned environmental conditions in the sea.

89. Effect of an insectcide, parathion, on aquatic animals in the lower range than its lethal concentration. Yoshiyuki MATSUE, Takuo ENDO and Kenji TABATA (Bull. Jap. Soc. Sci. Fish. **23**, 358–362, 1957)

Goldfish (*Carassius auratus*) ad Japanese killifish (*Oryzias latipes*) were kept for a long time, about a month, in water containing an insectcide, parathion (Diethyl-para-nitrophenyl-thiophane) in various concentrations lower than its lethal value and its toxocity upon fish was observed.

By the culture experiment it was known that the insectcide gape many recognizable effects upon fish even at low concentrations as 1/30th of its 48h-TLm (median tolerance limit), i.e., the movement of gill opercula and pectral fins of the fish was observed to decline until the above mentioned concentration of the insectcide. Retardness in growth of fish due to the decrease in the amount of food eaten was also apparent at these low concentrations.

The results show us that the permissible concentration of parathion upon aquatic animals should be regarded as far lower than the value of 48h-TLm$\times$0.1, which is now considered as might be permissible for general pollutants.

90. An ecological study on the euphausiids distributed in the Japan Sea. Yuzo KOMAKI and Yoshiyuki MATSUE (Rep. Invest. Exploit. Tsushima Warm Current Waters **2**, Fish eggs, fish larvae and plankton, 146–159, 1958)

The investigation for exploitation of the Tsushima Current Waters was carried out under the co-operation of Prefectural Fisheries Experimental Stations distributed along the coast of the Japan Sea, Regional Fisheries Research Laboratories and laboratories of universities, for five years since 1953. In this investigation we were assigned to the ecological study of euphausiids, which are the most important members of natural food for Japanese mackerel (*Scomber japonicus* HOUTTYN) and many other fishes.

From the results obtained by examining many thousands of samples collected by plankton net and larvae net, we conclude that the following four species are generally distributed in the Japan Sea.

Euphausia pacifica H. J. HANSEN
Pseudeuphausia latifrons (G. O. SARS) H. J. HANSEN
Thysanoessa longipes F. P. BRANDT
Thysanoessa inermis (K. KRÖYER) H. J. HANSEN

Discriptions of these species were made, and their geographical and vertical distribtuions were discussed, referring to their environmental conditions. The 'spine-less' form of *Th. longipes*, which was discussed by BANNER (1949), BODEN *et al.* (1955) and NEMOTO (1957) on the Pacific specimens, could not be found in our samples.

E. pacifica, the most important and widely distributed species among these euphausiids, has a peculiar characteristic of making so dense swarms in the coastal regions in spring, that the sea surface turns redish in colour. But, although extraordinary spring swarmings were seen in almost whole coastal waters of the Japan Sea during the period of 1947-1950, such phenomenon has never been observed after that. On the other hand, as this spring swarmings have been observed in the nearshore waters off Kinkazan every spring in recent years, we were able to trace the fluctuation in hte quantity of *E. pacifica* in this region, referring to the seasonal change of water temperature and variation of water temperature of the swarming period of each year. Then we ascertained the fact that the inflow of the cold offshore waters in early spring has the effect of changing the abundance of *E. pacifica*. Based on this fact, we discussed that the variation in water temperature at Kyogamisaki may be controlling the swarmings of euphausiids in Wakasa Bay.

91. The purification of radioactive sewage by activated sludge process. Masaaki MURANO and Yoshiyuki MATSUE (Industrial Water 4, 22–24, 1958)

The activated sludge process which is usually applied to the purification of domestic and industrial sewage has been tried at radioactive sewage treatment. Under several conditions we made the experiments on the absorption by activated sludge, using strontium-90 as synthetic radioactive sewage.

According to the differences in the condition of sample sludge, activated sludge proves to show diverse results in its uptake. It must be due to the differences of biological phases growing in activated sludge, to say nothing of the changes of external conditions. Activated sludge removes 70 per cent of radioactivity by aeration of 5 to 6 hours, and after aeration of 24 hours the removal, at the maximum, reached 96 per cent.

pH of activated sludge and the non-radioactive strontium mixed with radioactive sewage have great influences upon the removal by activated sludge. The removal, generally, brings more successful results on alkali sewage than acid sewage. The existence of nonradioactive strontium, even if its concentration is very thin so much as 1 ppm, produces unsuccessful results, compared with that of control. And calcium which belongs the homologous series of strontium also interferes the uptake by activated sludge.

92. Study of fish culture in an aquarium of closed-circulating system. Its fundamental theory and standard plan. Aritsune SAEKI (Bull. Jap. Soc. Sci. Fish. 23, 684–695, 1958)

This aquarium consists of fish tank, filter-bed, sediment-collector, circulating and aerating apparatus. The nitrogen and phosphorous cycles in the aquarium were investigated and the rate of secretion of metabolites, and the velosity of their decomposition were measured. And a method to determine culturing capacity and

the standard plan of the aquarium by this system was proposed.

The water of the aquarium contains much nitrate and phosphate which make the water acidic, but other elements of metabolites are scanty in quantity and in-nocuous to fish. So the necessary conditions in which the fish can be cultured healthly and normally are as follows:

(1) When M indicates dissolved oxygen, carbon-dioxide, ammonia and organic matter in the water at t time respectively, necessary conditions which enable the culturing for a short time are $dM/dt=0$. If aeration is enough, ammonia may be the index to satisfy these conditions, because it is more nocuous or poisonous and its oxydation is more difficult than organic matter. When nitrogen compounds secreted from the fish is F, oxidized ammonia by bacteria is X_1, assimilated nitrogen compound is X_2:

$$\frac{dF}{dt}=\frac{dX}{dt}+\frac{dX_2}{dt}$$

From the results of fish culturing experiments dF/dt is 50 mg per day per 100 grams of fish. Ammonia is oxidized by the action of bacteria developed on the filter-sand and this oxidation is monomolecular-autocatalytic in reaction. And its velocity is proportional to the ratio of exchangable ammonia of the sand to total ammonia of the system. Experiments with percolating apparatus and culturing the fish in the aquarium show that dX_1/dt is 0.14 mg per day per 10 g of the sand and dX_2/dt *is* 0.07 mg per day. The sand used in the experiments was 2-5 mm size of calcite or weathered granite. Therefore to satisfy these conditions 300 g of filter sand should be used for culturing 10 g of fish.

(2) Necessary conditions which enable the culturing for a long time without changng the water are to keep constant alkalinity and hydrogen-ion concentration. Some metabolites of the cultured fish are stocked in the water as nitrate and phosphate which precipitates with calcium or magnesium. As calcium or magnesium ions is eliminated from the water, it loses excess base and becomes acidic. This makes the fish unable to live and supresses the ammonia oxidation by bacteria. So the water of the aquarium has the need to neutralize the acidity to satisfy these conditions.

Table

(1) Weight of culturing fish, capacity of fish tank, volume of water and filter-sand as follows:

	Weight	Capacity	Volume of water
Culturing fish	×	×	—
Capacity of tank	1–20× (water)	1–20×	1–20×
Volume of sand	30×	10×	5×
Total	32-51×	12–32×	6–25×
% of fish to total	3–2	8–3	16–4

(2) Rate of circulation: once per 10–120 minutes.

(3) Velosity of filtration: 100–700 m/day.

(4) Rate of aeration: 2–3 times in volume of circulating water.

From these experimental results the standard plan of the aquarium by closed-circulating system is proposed as shown in Table. And to keep the water in a good condition for the fish, air and calcium-oxide or magnesia should be put in according to the analytical results of pH, dissolved oxygen and alkalinity of the water.

93. The culture of eel, *Anguilla japonica*, in the laboratory by sand-filtering and circulating system. Aritsune SAEKI (Aquiculture **6**, 36–42, 1958)

The eel and its larva (Shirasu) were cultured by the circulating and sand-filtering system. They were cultured in small cylindrical glass vessels of diameter 30-34 cm, depth 15-17 cm, and total culture water including the filter-bed was 20-30 litres. Eels fed with some *Gymnodrilus sp.* and the meat of the fresh-water or marine bivalves.

No loss of them were observed except in cases of accident or trouble (e.g. stoppage of electric current). The growth of the eels were normal and as good as that of other culture methods. The ratio of the increase in weight of the eel to given food weight, 16-20%, was similar to other culture methods. The body length of the eel grew up to 34 cm (mean value) which was the diameter of the culture vessel. The maximum weight of the cultured eel was 5% of the total aquarium water. These experimental results of culture and also the conditions of the aquarium water were what that was expected from the theory of this culture by circulating and sand-filtering system.

94. Choline content of the muscle of aquatic animals. Takajiro MORI, Shôji KÔNOSU and Shôji MIYAGAWA (Bull. Jap. Soc. Sci. Fish. **23**, 282–284, 1957)

The microbiological assay method of choline deviced by HOROWITZ was adopted after a slight modification. The total nitrogen was found to be applicable as an index of the growth of the test organisms, *Neurospora crassa cholineless mutant* No. 34486, instead of the dry weight, which had been adopted by the original author.

The muscles of several kinds of aquatic animals, such as yellowtail, bigeye tuna, jack mackerel, sardine, "nameta" flounder, common squid, octopus and hard clam, were determined by the modified method and the following results were obtained.

(1) The ordinary muscles of fish revealed a considerably constant level of total choline, ranging between 0.4 and 0.6 mg per g. on wet basis, but the level was two to three times higher in the red muscle of yellowtail and the deep-seated red muscle of bigeye tuna.

(2) Total choline contents of the squid and the octopus muscle were found to be 1.08 and 0.47 mg per g. respectively.

(3) Free choilne content, on the other hand, was very low in all the muscles of animals investigated, with the exception of fresh hard clam and stored squid, in the frozen state.

95. On the determination of trimethylamine and trimethylamine oxide. A modification of the Dyer method. Yoshiro HASHIMOTO and Tomotoshi OKAICHI (Bull. Jap. Soc. Sci. Fish. **23**, 269–272, 1957)

The DYER method on the determination of trimethylamine (TMA) and trimethylamine oxide (TMO) was examined and modified.

The extraction of TMA with toluene was found to be affected by temperature, sorts of alkali and number of shaking times, whereas the color development of TMA with picric acid was not influenced by temperature. From these observations, the extraction procedure was modified to be carried out at a definite temperature (30°C) and to shake for 60 times.

In the second place, the same volume of 25% potassium hydroxide was adopted instead of original 50% potassium carbonate solution, which was confirmed to be not suitable for the TMA solution resulting from the reduction of TMO with DEVARDA's alloy and hydrochloric acid. The carbonate forms white colloidal precipitates, perhaps carbonates of Al and Zn, in the aliquot of the heated reducing mixture, and the precipitates were suspected to adsorb or occlude TMA. The hydroxide does not give such precipitates.

The modification above-mentioned facilitated the determination of smaller amounts of TMO and the modified method gave over 97% recovery down to 0.025 mg N, which is far lower than 0.4 mg N in the DYER method. The recovery of TMO added to carp muscle extracts was also satisfactory.

96. Studies on the carotenoids of salmon. Part 1. Identification of the muscle pigments. Tsunetoshi KANEMITSU and Hiroshi AOE (Bull. Jap. Soc. Sci. Fish. 24, 209–215, 1958)

Absorption spectra and extinction coefficients of the crystalline astaxanthin and astacene prepared from lobsters and crawfish, were determined in various solvents. In chromatography, astaxanthin was almost pure, but sometimes separated into two fractions (Ax.-Fr. A and B), whereas astacene was usually separated into two fractions (Ac.-Fr. A and B). By the observation of absorption spectra of these fractions, it was assumed that Ax.-Fr. B and Ac.-Fr. B correspond to mono-cis isomer of Ax.-Fr. A and Ac.-Fr. A respectively, which are of all-trans form and commonly called "astaxanthin" and "astacene".

Using the crystalline pigments mentioned above as authentic specimen, identification of the muscle carostenoid of the North Pacific salmon was performed. The muscle was extracted with acetone and the extracted pigment was then transfered to petroleum ether, which was chromatographed on cellulose powder. The impurities mainly composed of lipids (Fr.-1) were not absorbed by the powder. The pigment adsorbed was easily developed and eluted from the column with acetone (Fr.-2). Fr.-2 was further chromatographed on $CaCO_3$. The results obtained show that the muscle of these salmon contained astaxanthin as the main pigment (Fr.-2-1) and a small amount of the pigment (Fr.-2-2) which was considered as mono-cis isomer of all-trans astaxanthin. Neither astacene nor β-carotene was detected.

97. Muscle extracts of aquatic animals. Part 1. Amino acids, trimethylamine and trimethylamine oxide in the muscle extracts of a squid, *Ommastrephes sloani pacificus*. Shôji KÔNOSU, Teruko AKIYAMA and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. 23, 561–564, 1958)

Seventeen amino acids in the alcoholic extracts of squid muscle were microbiologically determined before and after hydrolysis. Proline (479 mg % on wet basis) was the most abundant among the free amino acids and occupied about half of the total amino acids. Histidine (140 mg%), arginine (99 mg%), alanine (75 mg %) and glycine (42 mg %) were found to be present in a relatively high level.

After hydrolysis, most of the amino acids increased rather slightly, but the following amino acids, such as glycine, aspartic acid, glutamic acid, proline and lysine increased considerably.

The content of trimethylamine (TMA) and trimethylamine oxide (TMO) in the same muscle was also analyzed by the DYER's method and found to be 9.5 and 1133.9 mg % on wet basis respectively.

About fifty per cent of the extracted nitrogen have been accounted for as amino acid-N (25.1%), TMA-N (0.3%) and TMO-N (27.2%).

Part 2. Amino acids in the muscle extracts of prawn. Shôji KÔNOSU, Teruko AKIYAMA and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. **23**, 565–567, 1958)

Seventeen amino acids in the alcoholic extracts of prawn muscle were microbiologically determined before and after hydrolysis.

The pattern of free amino acid distribution in the prawn muscle differed considerably from that in the squid muscle. Glycine (1250 mg % on wet basis) was the most abundant, which occupied about half of the total free amino acids. Arginine (686 mg %), proline (230 mg %), serine (108 mg %), glutamic acid (65 mg %) and alanine (58 mg %) were present in a relatively high level. The content of aspartic acid was very low, as was observed in the squid muscle.

After hydrolysis, most of the amino acids increased slightly, but the following amino acids, such as glutamic acid, aspartic acid and glycine, increased considerably.

The recovered amino acid nitrogen in the analysis amounted to about seventy per cent of the extracted nitrogen, showing that amino acids are the main nitrogenous component of the prawn muscle extracts.

98. Chemical studies on the red muscle ("chiai") of fishes. Part 7. Electrophoretic characteristics of red muscle, myoglobin and blood hemoglobin. Fumio MATSUURA and Kanehisa HASHIMOTO (Bull. Jap. Soc. Sci. Fish. **23**, 222–226, 1957)

Metmyoglobin (MMb), prepared from the red muscle of tuna, *Parathunnus sibi*, after SCHMID, was analysed electrophoretically with a TISELIUS apparatus, and it was found that this preparation was not homogeneous except around the isoelectric point. In addition to the main component, a minute one was discerned, the mobility of which differed from that of tuna methemoglobin (MHb) at any pH and which was removed unsuccessfully by repeating recrystallization. Horse heart MMb, tested for reference, behaved in the same way at pH 6.0. It is uncertain at present whether this minute component is the denatured product from native Mb or not.

In electrophoresis of MHb's of tuna and horse, there were observed similarly plural components, the relative amounts of which were, however, fairly close to each other, differing from the cases of MMb's.

The electrophoretic mobility of tuna MMb is very small, at least in the pH

region covered in this experiment: e.g., in the phosphate buffer ($\Gamma/2$ 0.15, pH 7.3) the mobility of tuna MMb is approximately one half of horse's, one seventh of carp's, and one fifth of turtle's. The isoelectric point of tuna MMb was observed to be at pH 6.8, while that of tuna MHb at a far lower pH (5.8).

Part 8. Preparation of crystalline cytochrome c from the red muscle of tunny-fish. Fumio MATSUURA and Kanehisa HASHIMOTO (Bull. Jap. Soc. Sci. Fish. 24, 216–220, 1958)

Crystalline cytochrome c (Cyt) was prepared from the red muscle of tuna, *Neothunnus macropterus*, by the method of HAGIHARA with some modifications. The twice-recrystallized specimen was then analyzed for the iron content, spectral and electrophoretic properties.

The iron content of the preparation was found to be 0.487%, which is slightly higher than the values of the crystalline Cyt's from the other sources (0.43~0.47%). The spectral characters agreed fairly well with those reported on amorphous Cyt's except in the lower absorption coefficient for the minimum (about 535 mμ), which makes the ratios of maxima (550 and 520 mμ) to the minimum higher.

The electrophoretic experiments revealed the general resemblance of pH-mobility curve of tuna Cyt to those of mammals or chicken, although Cyt of the former moved more rapidly than the latter in the strongly acidic or alkaline pH range. The isoelectric point of tuna Cyt was found to be at pH 10.2, which is slightly lower than those of all the other species mentioned above (pH 10.7~10.9).

99. Studies on the radioactive materials in the radiologically contaminated fishes. Part 4. Group separation analysis of the radioelements in a contaminated big-eyed tuna, *Parathunnus mebachi*. Kazuo SHIRAI (Bull. Jap. Soc. Sci. Fish. 23, 723–728, 1958)

Group separation analysis using carriers was carried out on the radio-elements in liver, pyrolic caeca, kidney, red and ordinary muscles of a contaminated big-eyed tuna, *Parathunnus mebachi*, caught at a station (12°17′N, 167°04′E) in the Pacific Ocean on Oct. 20, 1956.

In the liver and the pyrolic caeca, group II had the major part of total activity, but group III B obtained from both organs or group IV from the liver also showed a considerably strong activity. The nuclides contained in group II were identified as Cd^{113m}, and Cd^{115m} by the further fractionation and the estimation of half life and energy of β-ray.

Radioactive elements in group III B and IV were recognized to be Zn^{65} and Sr^{90}-Y^{90} respectively, judging from the decay and absorption curves.

Similarly, radioactive materials contained in kidney were fractionated and identified as Cd^{113m} and Cd^{115m} in group II, as Zn^{65} in group III B, and as Sr^{90}-Y^{90} in group IV. Members of group III A were not thoroughly examined because of the disturbance of phosphate, although the group in the kidney, red and white muscles revealed a considerably high activity.

Part 5. Column chromatography of the radioelements in the liver of a big-eyed tuna, *Parathunnus mebachi*, with an ion exchange resin. Masamichi SAIKI (Bull. Jap. Soc. Sci. Fish. 23, 729–734, 1958)

The radioactive elements in the liver of the same tuna reported in Part IV has

been fractionated with an ion exchange resin (Dowex 50-X8). Mn^{54}, Sr^{90}, and Y^{90} were confirmed to be present in this organ together with the formerly identified nuclides, such as Zn^{65}, $Fe^{55,59}$, $Cd^{113m,115m}$, Ba^{140} and La^{140}.

Among these nuclides detected, Mn^{54} has been found for the first time as radioactive contaminant resulting from the atomic bomb tests.

The presence of Sr^{90} was also confirmed by the group separation method using carriers.

The quantity of the radioactive nuclides in 100 g of the wet tissue was calculated as follows: Fe^{55} (Fe^{59}) 0.5 μc, Zn^{65} 0.05 μc, Cd^{115m} (Cd^{113m}) 0.012 μc, Mn^{54} 0.0022 μc, Sr^{90} 0.002 μc, Y^{90} 0.002 μc and Ba^{140} and La^{140} trace. Special attention should be paid for the presence and the amount of Sr^{90}, judging from the maximum permissible dose given by the International Commission on Radiological Protection in 1954.

Part 6. Column chromatography of the radioelements in the pyrolic caeca of a big-eyed tuna, *Parathunnus mebachi*, with an ion exchange resin. Kazuo SHIRAI (Bull. Jap. Soc. Sci. Fish. 23, 735–737, 1958)

The ash of pyrolic caeca of the same tuna reported in Part IV, has been fractionated by column chromatography with an ion exchange resin (Dowex 50-X8, 100-200 mesh), and seven radioactive fractions (F 1-7) were obtained.

F 1 was further analysed by the group separation method using carriers and the greater part of activity was detcted in group III A. This group had a long half life and an extremely soft energy. These chemical and physical properties indicate that the nuclide in this fraction is Fe^{55}.

The absorption and decay curves of other fractions except F5 and F6 were obtained directly on the effluent, without further purification. These curves indicated that the nuclides in F2, F3, F4 and F7 were Y^{90}, Zn^{65}, a mixture of Cd^{113m} and Cd^{115m}, and Sr^{90}-Y^{90}, respectively.

The activity of F5 and F6 was very weak and short-living. These fractions were supposed to contain Y^{90} originated from Sr^{90} in the interruption of elution.

VI. Forestry

Abstract Nos. 100—114

100. An ecological study of may-beetles on the coastal sand dune. Part 4. Particle size compositions and white grub inhabitation. Masatoshi NITTO and Kanji TACHIBANA (Bull. Tokyo Univ. Forests 53, 151–159, 1957)

In this paper, we took up some relations between the particle size composition of sands and white grub inhabitation.

A total of 58 samples were taken mainly from the beach of Nishizaki to Kanbe, and some of them from farmers' yards in inland. Sands of the surface and from a 20 cm depth in each plot came into 6 classes according to the particle size by sieving and weighing them. What percentage a sample had in weight of each size, finally

classified the sand composition of this area into 9 types.

Studying each type of the sands, we found the grub-inhabiting plots restricted within the type C, C′, D, E. Characteristics of types are: large particles of sand on the surface, and the different sand composition of the upper and lower layer, probably owing to a great deal of sand displacement by the wind. We found few grubs in the Stable place, where similar composition of sands was in both layers and the particles were smaller.

101. Studies on the heartbeat of insect. Kanji TACHIBANA and Chikao NAGASHIMA (Jap. Journ. Appl. Ent. Zool. 1 [3], 155–163, 1957)

High skilfulness has so far been required to record the heartbeat of insect. The new method devised by the present authors made it much easier to record the heartbeat of an intact insect.

This method was based in principle on photo-electric effect: a constant beam of light was thrown on the heart of the dorsal vessel which was seen through the mid-dorsal cuticle. The changes in a reflected light associated with heartbeat were converted to changes of potential difference by means of the photocell, and were recorded as mechanocardiogram.

The action potentials were either led off from the cuticular surface of ether-anaesthetized larva by means of one pair of forceps-type electrodes or led off from the inside of ether-anaesthetized larva by means of one pair of needle-type electrodes, and were fed to the amplifier. Thus the action potentials were recorded as electrocardiogram.

Observations on the heartbeat by these two methods were made with both the last instar larvae of grubs, *Xylotrupes dichotomus* L., and those of the silkworm, *Bombyx mori* L.. The mechanocardiograms or the electrocardiograms observed with these insects were compared with those reported by other investigators.

102. Notes on Japanese ferns (13-16). Satoru KURATA (Journ. Geobot. **6**, 68–71, 111–114, 1957; **7**, 11–14, 39–43, 1958)

From the taxonomical and geobotanical view-points, critical notes are written on such ferns as *Athyrium subrigescens*, *Cornopteris hakonensis*, *Diplazium dimorphophyllum* form. *kiusianum* (stat. nov.), *Polystichum*×*Namegatae* (hybr. nov.), *P.*×*ongataense* (hybr. nov.), *Dryopteris*×*hakonecola* (hybr. nov.), *D.*×*Mayebarae*, *D. polita* (new to Japan), *Leptogramma mollissima* var. *pilosissima* (comb. nov.), *Polystichum Tagawanum* (sp. nov), and *P.*×*izuense* (hybr. nov.).

103. On the behavior of the vascular strands at the cotyledonary- and the first-node of the genus *Quercus*. Ken SHIMAJI (Bull. Tokyo Univ. Forests, **53**, 125–134, 1957)

Nodal anatomy of the cotyledon and the first leaf was studied on twelve representative species of the Japanese *Quercus*, as an investigation on the ontogenetically earlier stage.

The clear classification of the genus *Quercus* on the basis of the anatomy of adult parts became obscure and a completely different classification was established on the anatomy of the cotyledonary and the first-node.

With some discussions, the author was led to the conclusion that the ontogenetic

development of the nodal anatomy, even together with the above-mentioned dieffrence of classifications, much reinforce the phylogenetic relation among the genus *Quercus* which was suggested by the author on the basis of anatomical characteristics of adult parts previously.

104. Alpha-keto acids in pine seeds. Ken-ichi HATANO (Bull. Tokyo Univ. Forests 53, 135–138, 1957)

α-keto acids in pine seeds (*Pinus Thunbergii*) at the germination stage were analysed as 2.4-dinitrophenylhydrazones by paper chromatography of n-butanol saturated with 3% aqua ammonia.

Pyruvic acid and α-ketoglutaric acid were found at all phases, and they increased gradually with the germination process. Their rise and fall have no relation to chlorophyll building and photosynthesis. In this experiment, oxaloacetic acid was not detected at all under light or dark condition.

105. On the amino acids of *Paulownia tomentosa* (THUNB.) STEUD. affected by the Witches' broom. Part 1. The amino acids of leaves and twigs. Haruyoshi SAHO (Journ. Jap. Forestry Soc. 39, 447–451, 1957)

Paulownia tomentosa (THUNB.) STEUD. is one of the most useful tree in Japan. Recently, the Witches' broom, the most destructive disease of this tree has prevailed in this country and the cultivation of *Paulownia* tree seems to become difficult in future.

Some investigators studied the *Paulownia* Witches' broom from the physiological and pathological standpoints, and they reported that the *Paulownia* Witches' broom might be a virus disease. The tests on the preventing of the disease and the detecting methods of the symptomless carrier have been not accomplished.

The author studied on the amino acids of *Paulownia* tree to give a fresh aspect to the tree physiology and tree pathology of the *Paulownia* Witches' broom.

106. Wood decay of *Abies sachalinensis* forest in the Tokyo University Forest, Hokkaido. Part 3. On wood decay of Oni-todo. Shun-ichi YOKOTA (Bull. Tokyo Univ. Forests 53, 139–150, 1957)

It is well known that the color of the bark of *Abies sachalinensis* MAST. is grayish white. Often there appear, however, individuals having cracks as those of spruce or black protuberances in their bark. In many cases, they have such defects as thick bark and branches and bent trunk. These individuals are named **'Oni-todo'** or **'Oni-hada-todomatsu'** (*Abies sachalinensis* FR. *Schm.* var. *corticosa* TATEWAKI). According to the results of the survey of wood decay in 200 individuals of these **'Oni-todo'**, a consideration was given on the handling of **'Oni-todo'** in the stands, with respect to forest pathological aspects.

1. The frequency of occurrence of decay was very high in each diameter class. The facts that Fomitopsis annosa (FR.) KARST. was the fungus most active in destroying the butt and root, amounting to more than half of the total volume of decay, and the height of decay of this fungus was considerably high, were the same as those in the survey during 1953-55. Other fungi causing wood decay are *Poria subacida* (PECK) SACC., *Bondarzewia montana* (QUÉL.) SING., *Phaeolus Schweinitzii* (FR.)

PAR., *Tyromyces balsameus* (PECK) MURR., *Phellinus Hartigii* (ALLESCH. et SCHNABL) IMAZ. etc., among which *P. Schweinitzii* and *T. balsameus* were important because of their strong activity destroying the butt and root.

2. Because of the decrease of the mechanical strength of trunks by the heart rot, there is a danger predisposing to wind-throw. Since majority of **'Oni-todo'** are large, when the stands encounter to the strong wind and the wind-throw happens in them, young healthy trees will be crushed and many damages of the turning over will result.

3. According to the condition of growth of **'Oni-todo'** observed by comparing with that of normal Sakhalin fir grown in the same stands, it was cleared up in general that the growth of **'Oni-todo'** was poor. For example, the ages of **'Oni-todo'** of 25, 33.5, 36 and 47 cm D.B.H. were 150, 161, 140 and 167 years, respectively, while those of normal individuals of 23, 30, 35, 43 and 52 cm D.B.H. were 77, 123, 100, 142 and 90 years, respectively. The rate of growth in volume of **'Oni-todo'** was far smaller than that of normal trees in the same stands.

4. The butt and root heavily destroyed becomes the source of infection to the healthy trees.

Because of these circumstances, the writer considers that the trees having the characters of **'Oni-todo'** should be cut at the earliest opportunity to improve the forest condition.

107. Estimation on amount of water loss by transpiration of coniferous forest. Taisitiroo SATOO (Kagaku **28**, 205, 1958)

Amount of water transpired by the pure stands of *Cryptomeria japonica*, *Chamaecyparis obtusa* and *Pinus densiflora* was estimated. As shown in the table, the rank of species put by amount of water loss by transpiration of stands on the unit area differd from that by unit amount of leaf.

Species		*Cryptomeria japonica*	*Chamaecyparis obtusa*	*Pinus densiflora*
Amount of leaves in green wt. t./ha.		36.5	30	12
Rate of transpiration	Leaf per 1 g. g./day May~Oct.	1.31	1.29	2.92
	Leaf per 1 g. g./day Nov.~Apr.	0.52	0.47	1.07
	Stand per 1 ha. mm./day May~Oct.	4.8	3.9	3.5
	Stand per 1 ha. mm./day Nov.~Apr.	1.9	1.9	1.3
	Stand per 1 ha. mm./annum	1230	970	680

108. A methods for the rapid measuring of leaf areas. Ken'itiroo NEGISI, Taisitiroo SATOO and Kitokuroo YAGI (Journ. Jap. For. Soc. **39**, 380–384, 1957)

Leaf area is determined easily by placing a leaf randomly on a board with dots of given distances and counting the dots covered by the leaf. The authors named

this method "dot counting method". By this method determination of the areas of leaves *in situ* is made more easily and more rapidly than the other methods. The accuracy is dependent on the distances of dots on the board and the number of replications. This method is applicable for ordinary broad leaves and leaves of *Ginkgo biloba*, but thed veiation is too great for the measurement of the areas of the leaves of complicated shape such as *Thujopsis dolabrata*. Discussions are given for the accuracy, distances of dots, and replications.

109. Tractor logging operation for the utilization of timbers damaged by storm. Makoto FUJIBAYASHI, Shoji OKAWARA and Masayuki SUZUKI (Misc. Tokyo Univ. Forests **12**, 95–119, 1957)

In September 1954, a great deal of natural resources of Ezomatsu and Todomatsu (*Picea jezoensis* CARR. and *Abies Mayriana* MIYABE et KUDO) were destroyed by the enormous typhoon at Sōunkyo districts in Hokkaido. For logging out of these large amounts of the destroyed wood from the forest, tractor logging took place.

All tractors were crawler type: "Fordson 42 HP" (English made) and "CT—25 52 HP" (Japanese made). The tractive method of the former was "Pan way" and of the later was "Sulky way".

In August 1956, we authors had a chance to observe the logging operation and to investigate many problems about the tractor logging in our country. The results are summarized as follows.

1) To express the actual circumstances of the tractor logging by means of function of distance, no break-even-point was found between 200 and 1,000 meters, and the efficiency of "Pan way" was almost twice as advantageous as "Sulky way" but it was contrary in economy.

2) Advantage of "Pan way" in efficiency owed to the tractor logging alone. But in the "Sulky way", besides the tractor logging, tractor skidding was done without of "tree length logging". But as for the "tree length logging" we must study in future.

3) We can recognize the disadvantage of "Pan way" in economy. It was chiefly due to the use of horses in the skidding, which was easily influenced by the surroundings. And this often caused the irregular efficiency of the tractor transportation. So it is hoped to build the plan about the reliable method of skidding, and to decrease the cost of wood production.

4) Using several tractors at the same time in the timber yard, there often was overcrowding, which greatly caused to impede progress of work. The timber yard should be made wide or the disposition of tractor roads must be improved.

5) The efficiency of work of pan or sulky was chiefly influenced by the weather. After rain most tractor roads became muddy because of volcanic ashy soil, sulky tires often sank into the mud and rolling resistance increased, but in the case of the pan the running resistance decreased and a greater load might be admitted . After fine weather when the roads became dry these relations were contrary. There was little room for improvement in regard to the pan but there were many problems to improve the sulky so as to use under any disadvantageous conditions.

6) A load of tractor was $4.7 \sim 5.3\ m^3$ in the case of "Pan way", and $2.2 \sim 2.5\ m^3$ in the case of "Sulky way". Under the dry soil condition these figures were almost load limits.

7) This logging was done in the level forest, but if it were on a down hill, the efficiency of work would have to be greater than that and we should attempt to study on this subject in future.

110. On the lumbering operation by chain saw at the forest damaged by storm. Makoto FUJIBAYASHI and Shoji OKAWARA (Misc. Tokyo Univ. Forests **12**, 121–131, 1957)

From the previous results of several practical field operation tests of chain saw, the authors had an opinion that the lumbering by chain saw would take the place of conventional hand work in any forest of our country. Last summer, they had an opportunity to investigate about the logging operation of chain saw in the Hokkaido National Forest, which was seriously destroyed by the storm in order to utilize the large amount of the damaged timbers within a short period. The following results of this test also proved the propriety of this opinion.

1) Efficiency of work. Efficiency of work was influenced widely by the degree of the ability of workers, and in the case of moderate ability of workers, it was expected more than twice, comparing with hand labour.

2) Cost. Assuming the wages of workers remained in the usual official rate just as it was, the total expense of chain saw work would be nearly equal to that of hand work. But under this circumstance, the employer could shorten the net time of operation because of above consequence and on the other hand the employee could get wages more 700 yen per day than for hand labour.

3) Fatigue. The daily fatigue of workers was decreased in spite of heavy weight of chain saw, because of the short time of handling.

So we could conclude that chain saw work was more effective and useful especially for the purpose of exploiting a great deal of forest resource within a short period.

111. A preliminary study of the hydrologic year in Aichi. Yôichi NOGUCHI (Bull. Tokyo Univ. Forests **53**, 27–31, 1957)

The underlying assumption is that the period at which the water stored up in the ground is at a maximum could be definitely observed, the relation of rainfall to runoff for the interval between these maxima would be more constant than for any other period.

Stream gauging records on the experimental watershed in the Tokyo University Forest at Aichi have been analyzed. The ground water storage on this watershed has been found to be at its maximum about the end of April, and a balance sheet for ten hydrologic years including precipitation, runoff, daily flow, change of ground water storage and estimated evapo-transpiration has been derived. Based on these hydrologic years the annual runoff has been found to change linearly with more correlation to annual precipitation than for the water year which has been considered as most valuable up to this time.

112. Fundamental study on the measurement of discharge and analysis of hydrologic cycle phenomen on for watershed management. Isao YAMAGUCHI (Bull. Tokyo Univ. Forests **53**, 33–124, 1957)

To clarify water movement in drainage area is the basis for watershed management. This study is carried out for this purpose. The experimental data are ob-

tained from the Shirasaka watershed in the Tokyo University Forest, Aichi prefecture, where stream gauging has been carried on since 1929. Changes in topography and also in forest cover during this period are recognized to be of very little importance for the purpose of this study.

(I) Statistical consideration

a) Annual data

The runoff coefficient C. in the equation Q=C (R-A) (R=rainfall, Q=runoff, A=loss) shows such a slight decrease with years that it gives little significance.

b) Monthly data

As regards monthly amounts of rainfall (r) and runoff. (q) each is taken from the adjusted curve drawn on the graph showing monthly amount by month. Thus, the relation is obtained as

$$0.002\,r^2 = q - 20 \text{ (mm)}$$

It is also found that the runoff is affected by the last rain with considerable influences of those in the preceding 2~3 months.

c) Daily data

The standard deviation of daily runoff is discussed in relation to that of rainfall, resulting that the time influence cannot be seen on the daily runoff.

(II) Discussion of storm hydrograph

Using R.T. Zock's formulas $d\phi = rdt - qdt$ (ϕ=water contained in watershed) and $q = \phi$, storm hydrograph equations are derived involving the relation between area element and water travel time and also that between time and rainfall intensity. Finally the runoff coefficient c is discussed as being an important index, representing watershed characteristics. Thus, it is found that c varies according to rainfall intensity. For Shirasaka watershed the following results are abtained.

Rainfall intensity (mm/min)	Value of c
0 —0.1	0.0286±0.0114
0.1—0.3	0.210 ±0.096
0.3	2.00

The value of c for dry period is obtained as 0.00045—0.00006 and that for summer is three times as much as that for winter.

Apparent water loss in summer dry period is found as five to ten times as much as that in winter.

The writer presumes that the above loss is equal to the amount due to evaporation and transpiration from waterway and ground surface close to it.

113. A formula expressing the relation between annual rainfall and runoff. Sadao Ogihara (Journ. Jap. For. Soc. **40**, 244–246, 1952)

From the standpoint of practical application an experimental formula is expected to be simple in form and high in adaptability to observed data. Thus, the following types have been widely used: (1) $y=ax-b$ (2) $y=ax^b$ (3) $y=ax$ (4) $y=x-b$, where x=rainfall and y=precipitation. (1) and (2) have higher adaptability, but lower practicability in comparing two or more formulae. (3) and (4), on the contrary, show lower adaptability and higher practicabilit.

Here a new formula is presented as

$$Y^3 - X^3 + aY = 0$$

which has only one constant yet higher adaptability than (3) or (4), and also higher practicability than (1) or (2).

Moreover, the curve of this formula has a nature fitting to a theoretically presumable shape, which starts from the origin of the coordinates and never allows y to exceed x.

114. A study of channel-storage and its application to reservoir flood control. Isao YAMAGUCHI (The Tokyo Univ. Forests 12, 219–227, 1957)

In this paper, the auther deduced a theoretical solution on channal storage phenomena and also applied it to flood controlling effects of reservoirs. As the result, the following equation is derived.

$$\frac{Q_{t+\Delta t}+Q_t}{2}=\beta\Delta q_{\Delta t}+q_t, \qquad \beta=\frac{B\Delta L}{\Delta t}\frac{\Delta h_a}{\Delta h_{\Delta t_2}}\frac{1}{\frac{dq}{dh}}+\frac{1}{2}$$

where Q_t is inflow rate at time t; q_t is outflow rate at time t and given as a function of water depth; $\Delta q_{\Delta t}$ is the increment of outflow rate during the time Δt, h is the area of water surface and given as a function of water depth; he is the water depth of outflow.

By the preceding formula, we may estimate values of inflow rate from outflow rate.

VII. Forest Products

Abstract Nos. 115—135

115. A research on lumber for home construction. Shinji HIRAI, Kakuichi KITAHARA, Yasushi SUZUKI, Shitomo MAMADA, Yôji KIKATA and Yoshiyasu YAMAGISHI (Misc. Inform. Tokyo Univ. Forests 12, 245–298, 1957)

The utilization of wood and wood products in the field of home construction has been studied by statistically selecting 92 typical houses in Japan.

The results obtained are summerized under following headings.

1. Sizes of lumber used for each structural member.
2. Amount of lumber used for each structural member.
3. Amount of lumber used in unit area of home construction.
4. Amount of lumber of each size used in unit area of home construction.

116. Wane and knot in square cut from small logs of *Cryptomeria*. Nobuyuki EDAMATSU and Shinji HIRAI (Bull. Govern. For. Exper. Station 105, 187–218, 1958)

In Japan, square (scantling with square cross-section) production from small logs of *Cryptomeria* ("sugi", *Cryptomeria japonica* D. DON) at small scale sawmills is one of the most popular and important lumber productions. The mill study

described here consists of the estimates of wanes and knots which are considered to be the principal factors affecting the quality of such boxed heart square. Data were collected under ordinary working conditions at 12 sawmills, that were measurements of 2569 logs and lumber products recovered from logs.

1. Size of square from logs.

1) The diameters of logs range from 15 to 55 BU (1 BU=3.03 mm). They are most frequent at 35∼40 BU, and especially few at 55 BU and under 20 BU. Width of squares recovered from them range from 25 to 45 BU. They appear more frequently at 30, 33 and 35 BU, and comparatively few at 25, 28, 38, 40 and 45 BU.

2) There is a linear relationship between the log diameter and the average size of recovered squares.

3) An average lumber yield diagram from small logs was gained from this study.

2. Wane on square.

1) The wane on squares was measured on the basis of the measuring method of the Japanese Agricultural Standard (JAS). Wane was found anywhere in the part within 1/10 of lumber length from log ends on most of studied squares.

2) Effect of log and square sizes on the number of corners where wane is found in the part besides the vicinity of log ends is summarized in figure. From the figure, it is deducted that logs over 45 BU in dameter would be necessary to recover 30∼35 BU squares without wanes over in any corner.

3) Wane per cent of the sum or of the largest in one corner decreases with log diameter and increases with square size.

4) There is a correlation between the wane per cent and the "one corner ratio per cent", that is, the ratio per cent of the largest wane per cent in one corner to the wane per cent of the sum. This seems to indicate that the wane is more concentrative on one corner in high-quality classes than in poor-quality classes.

3. Knot on square.

1) The knot on squares was measured by an almost similar measuring method with that of JAS. Knots were found on all faces of squares, more than 90%, and squares with any clear face were only 5%.

2) There are some deviations between values for knots on squares from studied logs as to localities.

3) There is no clear correlation between the log size and the mean value for knots on the basis of all the studied logs. This tendency is also found even for squares from logs in one definite locality.

4) It might be accepted that there is an inverse linear relationship between the size of squares recovered from logs in one definite locality and the mean value of knot per cent and the knot number ratio (ratio of the number of knots to the unit width of square) on the most or least knotty face.

5) The mean value of knot number ratio is in linear relation to the knot per cent.

4. Observations on JAS grades for wane and knot on squares.

Some observations and comments were given on the JAS grades for wane and knot on squares from the results of this study. The authors also proposed a

tentative grading method for wane and knot on squares cut from small logs of *Cryptomeria* from a viewpoint of rationalization of standard.

117. Stress relaxation of chiphoard in hotpress. Kakuichi KITAHARA (Ball. Tokyo Univ. Forests 53, 161–177, 1957)

In chip-board production it is most desirable to press chip-board as soon as possible from the standpoints of both mass production with short cycle time of hot-press, and giving hard surface layers to board. But sufficient capacity of hot-press cannot be given generally for high specific gravity, on account of economical and technical difficulties, and further some time is needed for stopper (distance piece) to touch the upper hot plate of hot-press. The time is affected by specific gravity of board and its tendency is evident when specific gravity is much more than 0.70: it is infinitive when the figure is 0.8, meaning that stoppers do not touch the hot plate of hot-press continuously, and the thickness of board does not reach the estimated value in the case of hard wood raw material.

Hard surface layers and low density central layer may be made when the board is pressed in a very short time to a certain specific gravity. Surface layers that are fairly hard may be able to give good physical nad mechanical properties to board, consistent with the mass production having short cycle time of hot-press, for pressing progress is one of the narrowest paths in this production.

A rather low specific gravity of board is desirable for both manufacturing and use in actual practice, as a high specific gravity board is hard to cut and handle and a tendency to warp.

Strsess relaxation affects not only the time of stopper touching the upper hot plate of hot-press, but also the step-down stage of hot-press pressure and thickness of board. Compression shrinkage by decrease of water content is larger when the stress relaxation comes out early, and pure drying time in which there is no stress relaxation is too long in hot-press. Spring-back after hot-press is large when board is taken out from hot-press before there remains residual stress in the board.

Consequently, it is very desirable to take out board from hot press just at the time when the compressive stress of board has relaxed completely to ensure making the thickness of board equal to the estimated value, because it becomes thicker than that of stopper by spring back after hot-press, and thinner by compression shrinkage in hot-press as above mentioned.

In short, the time at which stress of board has relaxed completely in hot-press must be known in practice, as it is affected by every condition of manufacturing, among them species of raw wood, dimensions of chips, water content, resin content, specific gravity, thickness of board, closing time to maximum pressure of hot-press and etc.

The relaxations between pressure of hot-press and pressing time of board which is made of hard-wood and has two surface layers and one central layer are obtained in both the cases of high pressure (13 kg/cm^2) and low pressure (5 kg/cm^2).

In these cases, the pressure of evaporated water in board exerts a minus action on stress relaxation. Its value cannot be ignored, especially when specific

gravity and water content are high. The relationship between velocity of stress relaxation and water content is indicated by minimum curve for the evaporated water evidently disturbs stress relaxation when water content is high, and mechanical properties of chips are superior when it is low.

The thickness of board decreases with compression shrinkage caused by decrease of water content after stress relaxation has finished completely. The value is larger when specific gravity is low and time of stopper touching upper hot plate of hot-press is shorter, viz. stress is small at first stage, its rexation comes out early and compression shrinkage occurs for a long time.

Briefly stated, it is desirable to have a hot-press which has a suitable capacity to shorten the time of stopper touching upper hot-plate of hot-press, and capable of giving hard surface layers to board. Secondly it is desirable that board has a rather low specific gravity, as stress relaxation comes out earlier and temperature raising is comparatively easy at central layer. It is very advantageous also from the standpoint of second working, so long as it has comparatively hard surface layers and proper strength (modulus of rupture, adhesive strength and etc.) for practical use.

118. On chipboard sawing by circular saw blade. Yôji KIKATA, Chikao WATANABE and Shinji HIRAI (Journ. Japan Wood Research Soc. 4, 75–80, 1958)

This report describes tests on sawing chipboard with several types of circular saw blades.

Tests are made of the effects of spindle speed and feed speed on electric work, electric power required, and quality of sawn surface.

The following results are recognized:

1. As the spindle speed is increased and the feed speed held constant, the work and power requirements are increased.

As th feed speed is increased and the spindle speed held constant, the work is decreased, but the power is increased.

2. The work and power requirements by sawing with various saws are found to depend almost entirely on the specific gravity of chipboard.

3. By sawing at a constant sharpness angle, the work and power are minimum at a hook angle of 15°. The above-mentioned tendency is more conspicuous when the specific gravity of chipboard is greater and the feed speed is higher.

4. When the tooth pitch is doubled, the work and power are decreased.

5. In relation to the saw blade height, above the table, the work and power vary as a minimum curve.

6. The roughness of end-surface of chipboard was measured by a simple apparatus and indicated by mean roughness.

As the feed speed is increased, and as the saw blade height above the table is increased, the roughness of sawing end-surface is increased.

Roughness value seem to be influenced a little by methods of fixing the saw.

119. Puncture test in plywood and hardboard. Shitomo MAMADA, Hideo KAKEGAWA and Shigeo YAMANA (Wood Industry 13, 298–303, 1958)

To obtain basic data for the puncture strength of plywood and hardboard,

experiments were made concerning a few items.

The experimental apparatus is shown in Fig. 1 in which the striking block continues to fall on the test board with increasing height of 5 cm till the board breaks.

The obtained results are as follows:

1. When the striking block repeatedly fell from the same height to break the board, the relation between number of strikes and falling height is shown in Fig. 2.

2. The relation between puncture energy [weight of striking block×height kg·cm)] and thicknes of test board is characterized by a nearly straight line.

3. The puncture energy increased as the radius of point of circle of striking block as increased.

4. In the relation between puncture energy and water absorption of test board, the puncture energy increased as the time of immerse in water was increased.

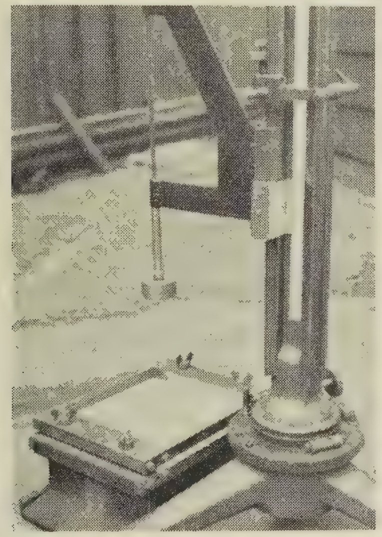


Fig. 1. Experimental apparatus for testing of puncture strength

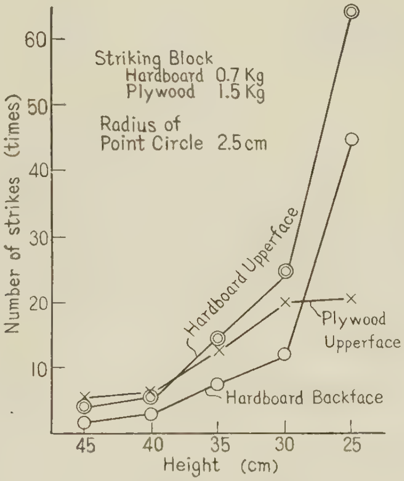


Fig. 2. Relation between number of strikes and falling height

120. On the properties of board affected by resin content of each layer in multiple chipboard. Shitomo MAMADA and Hideo NAKAJIMA (Wood Industry **12**, 471–473, 1957)

Lately, wood manufacturers have been interested in the development of chipboard industry, and consequently a considerable amount of investigation have been done in this field.

The resin content of chipboard plays an important part in point of the cost and the mechanical properties. In this experiment, the resin content of board (three layers chipboard) was kept in constant—about 9% based on the initial dry weight of chips—and the ratio of the resin content in each layer was varied (Table 1).

The dimension of the chips for surface was 0.2-1.0 cm length, 0.05-0.3 cm

width and 0.01～0.05 cm thickness, and for core was 3.3～55 cm, 0.3～0.5 cm and 0.05～0.1 cm respectively.

The woods used for surface were Ezomatsu (*Picea jezoensis*) and Todomatsu (*Abies Sachalinensis*), and for core was Shirakanba (*Betura Tauschii*).

The binder used was urea-resin, and sprayed by gan after adding water of about 2 times.

The weight ratio between surface and core chips was 1 : 1.

Condition of pressing was as follows;

Temperature; 145°C±5°C, presure and pressing time; 5 minutes on 15 kg/cm², 5 minutes on 10 kg/cm² and 10 minutes on 5 kg/cm².

After one week, mechanical properties were tested, and results obtained were as follows;

The bending strength, screw holding power and BRINELL hardness were increased with increasing of resin content in surface.

The delamination resistance showed a decrease as the resin content in surface were increased.

The principal conclusion of this study was that the resin should be sprayed little more on surface than on core.

Table 1. Resin and water in each layer.

Test-board No.		1				2				3				4			
Kind of layers	Weight of chip (g)	Resin		Water		Resin		Water		Resin		Water		Resin		Water	
		g	%	g	%	g	%	g	%	g	%	g	%	g	%	g	%
Surface	450	64	14.0	100	22.0	48	10.5	75	16.5	32	7.0	49	11.0	16	3.5	25	5.6
Core	450	16	3.5	25	5.6	32	7.0	49	11.0	48	10.5	75	16.5	64	14.0	100	22.0
Total	900	80	8.9	125	13.9	80	8.9	124	13.8	80	8.9	124	13.8	80	8.9	125	13.9

121. Studies on wood finishing. Part 3. The effect of filler on smoothness of paintfilm. Yoshihiko FUKUHARA (Wood Industry **12**, 421–425, 1957)

Previously the author investigated adhesion of paintfilm on wood surface and reported that filler padded only in vessels produced no remarkable reduction in adhesion.

The primary function of filling on wood finishing is the effect on surface smoothnes. So he studied in this report the effect of filling on the smoothness of painted wood surface by means of the optical-slit method. Species used is Shioji, *Fraxinus commemoralis* KOIDZUMI.

Smoothness was measured on the test surface before and after filling and after painting continuously. The following results are recognized;

(1) The application of filler improves the smoothness of painted surface. The filler at high viscosity gives good effect on smoothness. And the more times of filling are, the more smoothness is gained. But when refilling is applied on the still wet surface after the primary filling, the effect is not clear.

(2) Polishing after filling is useful for the improvement of surface smooth-

ness, but this polishing partically produces clear acute holes and raisings on the filled surface.

(3) The surface painted by shellac varnish is more smooth than the lacquer coating surface.

122. Studies on water-borne wood preservatives. Part 1. On the commercial cresols and their nitrated products. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Japan Wood Research Soc. 4, 46–50, 1958)

Wood preservatives injected into wood as water solutions have been much discussed since the late nineteenth century. The relation between the composition of the preservative and the characteristics of treated wood has been of a central interest, and many workers have investigated this problem. As a matter of fact, however, rather little is known about the Malenit, one of the fluoride-dinitrophenol preservatives (F.D.-type preservative) of Wolman salts.

The output of F.D.-type preservatives in Japan is shown. In this paper the compositions of commercial cresols and the analytical method of dinitrophenols contained in Wolman salts are studied.

1) The composition of five samples of commercial cresol produced by the Japanese tar industries were analyzed by the method of precise fraction, cineole method, Raschig's method, tolyloxyacetic acid method and by paper-chromatography.

2) Dinitrophenols were obtained by nitration of phenol and isomeric cresol which were sulphonated previously, and for the purpose of discerning dinitrophenols contained in commercial preservatives, infrared absordption spectra of dinitrophenols were measured. It seems that dinitrophenols contained in the Wolman salts can be analyzed by infrared absorption spectra between 12 and 14 micron.

Part 2. Determination of fluorine with thorium nitrate solution. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Japan Wood Research Soc. 4, 106–110, 1958)

The determination of fluorine is important not only for the inspection of commercial wood preservatives in which fluorine is contained, but also for the confirmation of fluorine penetration of treated wood. The analytical method for fluorine in wood preservatives containing sodium fluoride and dinitrophenate is described in JIS K 1550, but this method is calcium flouride gravimetric, and is neither simple nor accurate. According to Willard and Winter, fluorine may be titrated quantitatively with thorium nitrate in an aqueous solution.

We ascertained that the method of Willard and Winter was applicable and more suitable for the determination of fluorine in fluoride or in treated wood. Comparing the method of calcium fluoride gravimetric analysis with the method of thorium nitrate volumetric analysis, the latter possesses greater advantage in simplicity, applicability, and accuracy. As to the percentage of sodium fluordie, there is scarcely any recognizable difference existing between the two methods with thorium nitrate of titration without distillation and of titration after stream-disillation.

An apparatus for weathering test of treated wood is shown. Using this apparatus leached amount of fluorine and residue of fluorine in wood were determined, and fluorine in wood poles treated with 1.25% Malenit solution were also determined.

Part 3. On the significance of the antimony fluorine double salt contained in the wood preservative, sodium fluoride-dinitrophenate. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Japan Wood Research Soc. 4, 111–117, 1958)

As to the water-borne wood preservative, it is clear that in addition to the high toxicity, the permanence of the preservative is of great importance, that is to say, it must not be easily leached out by water or be rapidly evaporated, nor may it become easily inactive through oxidation, polymerization or other chemical changes. Wood preservative Malenit, developed by Malenkovic, contains an antimony fluoride double salt as an essential ingredient to control the corrosive action of the solution of sodium fluoride and dinitrophenate mixture, and to control the leaching out of dinitrophenate. But as to the leaching control of dinitrophenate, it has not yet been investigated. In order to determine and verify this point, the authors carried out the quantitative determinations of sodium 4,6-dinitro-*o*-cresylate remaining in filter paper, wood powder and wood piece respectively, after leaching out by water.

Morevoer, the metal corrosive control action of antimony fluoride double salt was determined.

The results obtained were as follows:

1) Some antimony fluoride double salts were prepared. Any marked influences of light and air on the precipitation from the aqueous solution of antimony fluoride double salts were not observed. In all cases, more than 90 percent of antimony was precipitated from the aqueous soltion of antimony fluoride double salts during 26 days, but fluorine was not precipitated.

2) The relation between solubility of sodium 4,6-dignitro-*o*-cresylate and pH of its solution was examined, and maximum absorption spectra of nitrophenols in visible region were measured.

3) The fixing ability of antimony fluoride double salts for 4,6-dinitro-*o*-cresylate were determined with filter paper, wood powder and wood piece.

Sodium 4,6-dinitro-*o*-cresylate was determined by colorimetric method. About the control ability in dinitro-*o*-cresylate leaching among the kind of tested antimony fluoride double salts, remarkable effects were not observed.

4) As to the metal corrosive control ability of antimony fluoride double salt, no significant difference could be observed.

123. Über die durch Vorbehandlung mit verdünnter Salzsäure aus Holzmehl gewonnene Lignine und die durch Abbau des Lignins entstehenden Substanzen in der Vorbehandlungslösung. Toshio FUKUZIMA and Takeo SHIBAMOTO (Journ. Japan Wood Research Soc. 4, 15–22, 1958)

Nach der Extraktion von BRAUNS-Lignin (Ausbeute 0.3%) wurde das Holzmehl der Ajan-Fichte (*Picea jezoensis* CARR.) mit 0.5 N wässeriger HCl Lösung behandelt, später mit Natronlauge neutralisiert und dann das Lignin durch Extraktion mit Aceton-Wasser gewonnen. Die Farbe des Lignins war schwach rotbraun und die Ausbeute berug 0.1% gegenüber dem Trockenholzmehl. Weiterhin wurde das Holzmehl mit 1 N HCl-Lösung behandelt und neutralisiert und dadurch graues Lignin durch heisse Extraktion mit Aceton-Wasser gewonnen. Die Ausbeute betrug 0.07%. Die Ultraviolett- und Infrarot-Spektren dieser Lignine waren dem Spektrum des

BRAUNS-Lignins sehr ähnlich. Der Methoxyl-Gehalt dieser Lignine steigt langsam an bei der Extraktionsfolge, wie in der Literatur von STUMPF und FREUDENBERG gezeigt wird. Und das Ultraviolett-Spektrum des 0.5 N HCl vorbehandelten Lignins hat keine Asborption auf dem Gebiet 340 mμ.

Nach der Extrakton von BRAUNS-Lignin wurde das gleiche Holzmehl mit 0.5 N HCl und 1 N HCl Lösung behandelt und neutralisiert, dann jedes Holzmehl mit Aceton-Wasser extrahiert. Die Ausbeute des Lignins bei 0.5 N HCl-Vorbehandlung war etwas besser als bei 1 N HCl-Vorbenhandlung.

Die Vorbehandlungslösung wurde mit Methylenchlorid extrahiert, und die Methylenchlorid-Lösung unter Stickstoff bis auf eine kleine Menge eingedampft. Ein Tropfen dieses Extraktes wurde auf Chromatographpapier übergetragen und in mit Benzol gesättigem Wasser entwickelt. Der weissblau fluoreszierende Fleck (R_F 0.37) war unter Ultraviolettlicht auf dem getrocknetem Papier nachweisbar und identisch mit dem Fleck con Coniferylaldehyd. Weitere unbekannte Substanzen (R_F 0.55 und 0.80) konnten durch Diazobenzolsulfonsäure-Farbrektion auf Papierchromatogramm gefunden werden. Es handelte sich dabei vermutlich um Guajacylaceton ähnliches Substanz (F_F 0.80) und Dimere von Guajacylpropan-Körpern (R. 0.55).

124. On carbonization of wood. Part 15. The thermal decompositions of cotton cellulose and cellulose acetate. Kyoji MINAMI, Hirotake ORII and Kimie KAWAMURA (Journ. Jap. Forest. Soc. **39**, 474–479, 1957)

The destructive distillations of cotton cellulose and cellulose acetate were carried out and the difference between intact wood and wood treated with 1% NaOH on their thermal decomposition was compared wth the present two materials. No report on the destructive distillation of cellulose acetate was found in literature, so far as the authors have been able to trace.

The yields of acetic acid on the destructive distillaton of cotton cellulose were very close to each other and, in total acid, 3.7% and, in volatile acid, 2.3%.

As for the course of thermal decomposition, in the case of cellulose acetate, maxima of the amount of distillate, the acid concentration and the amount of acid were coincidentally found at the fraction 200—220°C, and in the case of cotton cellulose, no maximum was found in this position. The relation between these two cases resembles that between an intact wood and the wood treated with 1% NaOH. Then, the accumulated quantity of acid at 260°C was, in the case of cellulose acetate, in total acid, 34.55% of its whole distilled amount and 34.93% in volatile acid and, in the case of cotton cellulose, 1.62% in total acid and 1.75% in volatile acid. This dierffence corresponded to the difference between an intact wood and the wood treated with 1% NaOH. These phenomena show that the distilling out of acetyl groups is the source of maxima in the neighbourhood of 240°C in the case of an intact wood, and also support the contention that acetyl groups in wood are the main source of distilled acetic acid in wood distillation.

Part 16. On the bondings of hemicellulose with other constituents of wood. Kyoji MINAMI (Journ. Jap. Forest. Soc. **40**, 16–26, 1958)

By the application of oxidative cleavage of glycol with periodate, an investiga-

tion on the bondings of hemicellulose with other constituents of wood was carried out. As materials for the periodate oxidation, woods free from alcohol-benzene extractive, woods treated with 1% NaOH, holocelluloses and holocelluloses treated with 1% NaOH were used to gain information on the bondings of hemicellulose with lignin and cellulose and the branching of hemicellulose. In respect to the kind of tree, *Quercus glauca* and *Mallotus japonicus* as broad-leaved trees, and *Cryptomeria japonica* and *Pinus densiflora* as coniferous trees, were selected at random.

The difference of the quantity of residual xylose after the oxidation between wood free from alcohol-benzene extractive and wood treated with 1% NaOH showed the quantity of xylose protected from the oxidation by its bonding with acetyl groups. In the case of broad-leaved trees, it was known that, if xylose and acetyl group were bonded at the ratio of 1 mol : 1 mol, 50%, at the least, of the whole acetyl groups in wood was combined with xylose or hemicellulose and, inversely, 50%, at the most, of the whole acetyl groups in wood was combined with constituents of wood other than hemicellulose, principally with cellulose. When the observed value of residual xylose amount in oxidized holocellulose and its calculated value on the acetyl content in holocellulose were compared in Table 3, the former was somewhat less than the latter, which means that a considerable amount of acetyl groups corresponding to the difference between the two values is bonded with constituents other than xylose. Further, the quantity of xylose protected from the oxidation by the bonding of xylose with cellulose and the branching of hemicellulose must be included in the observed value of residual xylose amount, and, therefore, the real amount of xylose protected by the bonding with acetyl groups should be less proportionally to the amount of xylose protected by the former.

In the case of coniferous trees, the acetyl contents of woods were low and, further, the small amount of acetyl groups, 33.6% of the whole acetyl groups for *Cryptomeria japonica* and 9.5% of the whole acetyl groups for *Pinus densiflora*, was bonded with xylose or hemicellulose. On examining the values, it was manifested that the observed values were not beyond the deviation limit of error and no distinct inference on the protection of oxylose from the oxidation was derived from the results. At any rate, it may be said that more quantity of acetyl groups than that in the case of broad-leaved trees is bonded with constituents of wood other than hemicellulose, principally with cellulose.

On comparing the quantity of residual xylose after the oxidation between wood free from alcohol-benzene extractive and holocellulose, a notable fact was found that, in all kinds of trees, about 20% of the whole xylose in wood is bonded with lignin.

The quantity of residual xylose in the oxidized hemicellulose of wood of *Quercus glauca* indicated that about 2.8% of hemicellulose was protected from the periodate oxidation by its branching.

As for the bonding of hemicellulose with cellulose, no distinct information was gained because the experimental values responsible for the bonding between them were within the deviation limit of error.

Part 17. Thermal decomposition of hemicellulose. Kyoji MINAMI and Kimie KAWAMURA (Journ. Jap. Forest. Soc. **40**, 62–67, 1958)

As for th destructive distillation of wood, an idea has widely prevailed that the most part of distilled acetic acid in wood distillation comes from hemicellulose. This has been conceived not only under the presumption that acetyl groups in wood are bonded with hemicellulose, but also has been often supported by the thought that much of the acetic acid is produced from the carbohydrate skelton of hemicellulose. As for the former, one of the authors, MINAMI, approved in the 16th paper of this series* that a quantity of acetyl group is bonded with hemicellulose in woods of broad-leaved trees as was suggested in past literature. The reality of the latter was examined in the present paper by carrying out the destructive distillation of hemicellulose extracted from the holocellulose of wood of *Quercus glauca*. The thermal decomposition of hemicellulose isolated by the method mentioned above has never been executed in the past.

As results of experiments, the following facts were known. The hemicellulose was decomposed by heat at lower temperatures as was stated in past literature: That is, to 260°C, the accumulated amount of distillate was 87.2% of its total distilled amount and the accumulated amount of acid, 86.5% of its total amount in total acid and 89.1% of its total amount in volatile acid. The acid concentration and the amount of acid in a unit fraction were high and large below 280°C. These phenomena were in distinct contrast with those in the case of cotton cellulose. Therefore, this remarkable decomposition of isolated hemicellulose at lower temperatures seemed unreliable as a foundation for the presumption of the behaviour of hemicellulose in wood on destructive distillation of wood. Then the calculation of accumulated amounts of distillate and of acid to 260°C for the holocellulose treated with 1% NoOH was tried, using the observed values of the amount of acid for the hemicellulose and the cotton cellulose and the composition of the material, and it was found that the calculated values had a good approximation to the actually observed values in the case of the holocellulose treated with 1% NaOH. So, it may be expected that the observed behaviour of the isolated hemicellulose on thermal decompositionare not far different from that of hemicellulose in wood on thermal decomposition.

The yield of acid from the hemicellulose was close to that from the cotton cellulose, and this seems to be reasonable in view of the resemblance of chemical structure as a carbohydrate between them, while the idea that hemicellulose itself is the main sources of acetic acid in wood distillation is denied.

Part 18. Thermal decomposition of wood celluloses. Kyoji MINAMI, Kimie KAWAMURA and Nagayoshi OSHIMA (Journ. Jap. Forest. Soc. **40**, 68–79, 1958)

The present communication is separated into two parts. In the first part, the thermal decompositions of celluloses, isolated by means of different methods, from the wood of *Quercus glauca* are reported, and in the second part, the thermal decompositions of celluloses of different origins are explained.

In Part I, as no communication on the thermal decomposition of holocellulose, which is the residue of wood extracted mainly lignin, was found in literature, the holocellulose was subjected to destructive distillation and the holocellulose treated with 1% NaOH, acetyl groups being removed off, was destructively distilled. Further, since the experiments on wood cellulose reported hitherto were carried

out using CROSS-BEVAN cellulose as a material, the CROSS-BEVAN cellulose from the wood of *Quercus glauca* was also decomposed by heat and, in addition, the CROSS-BEVAN cellulose treated with 1% NaOH too.

The decomposing course by heat of the holocellulose with much of acetyl groups resembled that of the intact wood and the treatment with 1% NaOH on holocellulose brought about the disappearance of the peaks of the amount of distillate, the concentration of acid and the amount of acid near 240°, similarly as the treatment with 1% NaOH on wood did. Thus, another proof was found here for the authors' deduction that a quantity of acetic acid comes from acetyl groups in wood in wood distillation.

The decomposing course y heat of the CROSS-BEVAN cellulose with less of acetyl groups rather resembled that of the wood treated with 1% NaOH, and the peaks of the amount of distillate and the amount of acid near 240° were not found. The yield of acid was somewhat more than the values found in literature, as shown in Table 1, but, if the acetyl content (as acetic acid) is reduced from the present value, the resultant remainder value is very close to the yield of acid for cotton cellulose observed by the authors under the allowance of experimental error. As the CROSS-BEVAN cellulose treated with 1% NaOH contained a quantity of pentosan (cf. Table 2), the close resemblance of the yield of acid between it and the cotton cellulose which contained almost no pentosan pointed out an incorrectness of the idea that hemicellulose is the main source of acetic acid in wood distillation. Thus, the differences of the yield of acid discovered by KLASON et al between cotton cellulose and wood celluloses and between conferous tree celluloses and broadleaved tree celluloses may be due to the difference of acety content between the materials.

In Part II, on th presumption that the difference of the yield of acid among celluloses frm different origins was possibly due to the difference of acetyl content in those materials, and in order to adjust the observation by KLASON et al, the residue of holocellulose made free from hemicellulose and treated with 1% NaOH, being considered as the purest wood cellulose, was destructively distilled for *Quercus glauca* and *Cryptomeria japonica.* The results showed that both of their decomposing courses and their yields of acid were quite accordant with those of cotton cellulose within the deviation of experimental error and so, it may be said that, whatever the origin of cellulose may be, it furnishes the same result on destructive distillation so far as it is pure.

As conclusions, it may be approved that in thermal decomposition the amount of acetic acid produced from cellulose is 3.6% of it, from hemicellulose 3.3% and from lignin 0.6%, and the difference between the observed yield of acid in wood distillation and the calculated yield of acid on the composition of wood and the yield of acid mentioned above for every component of wood, is corresponding to the content of acetyl group in wood.

125. Studies on pigments in fungi. Part 2. On the distribution of thelephoric acid in fungi (2). Maki SAWADA (Journ. Jap. Forest. Soc. **40**, 195–197, 1958)

Thelephoric acid was obtained from the fruit-bodies of two species of genus *Calodon* (*C. cyathiforme* (SCHAEFF. ex FR.) QUÉL. and *C. nigrum* (FR.) QUÉL.)

and of *Sarcodon amarescens* QUÉL.. This substance was found for the first time in the fruit-body of *Phlebia strigoso-zonata* (SCHW.) LLYD belonging to genus *Phlebia*.

126. Research on the swelling of fibers.Pa rt 3. Relation between the morphological structure and the behavior of swelling of fibers (1). Hayao HOSOI, Kiyoshi KATAHARA and Nobuhiko MIGITA (Journ. Japan Wood Research Soc. 4, 60–66, 1958)

Five samples of chlorite holocelluloses with different hemicellulose contents were immersed in phosphoric acid of several concentrations. In this treatment, fibers rich in hemicellulose give typical balloon swellings, while fibers with low hemicellulose content give typical spiral swellings.

It is supposed that the bands surrounding the surface of swollen fibers consist of fibril or a bundle of fibrils of the outer layer of the secondary wall. A crossed lameller structure exists in the outer layer of the secondary wall, namely, an outer Z-type, and an inner S-type.

Both the morphological structure and the hemicellulose content affect the swelling of fibers, and the former seems to be generally the dominant factor.

Part 4. Relation between the morphological structure and the behavior of swelling of fibers (2). Hayao HOSOI, Hajime TANI and Nobuhiko MIGITA (Journ. Japan Wood Research Soc. 4, 66–71, 1958)

In this paper, for the purpose of examining the characteristic behavior of swelling on hydrolysed fibers, dissolving sulphite pulp, dissolving sulphite pulp treated with 0.5% sulphuric acid, and dissolving sulphate pulp were swollen in phosphoric acid of varied cencentrations.

If the outer layer of the secondary wall was affected by acid, ladder-like bands arose. If the outer and the middle layers of the secondary wall were affected, disk-like particles arose.

It seems that the transverse morphological structure, easily hydrolysed by acid, exists in the outer layer of the secondary wall.

127. The behaviors of mercerized cellulose during emulsion xanthation. Nobuhiko MIGITA, Osakazu NAKAO and Akito KUDO (Journ. Soc. Textile and Cellulose Ind., Japan 13, 447–453, 1957)

In order to investigate the behaviors of mercerized cellulose in a course of emulsion xanthation, linter and rayon wood pulps and their mercerized pulps were emulsion xanthated under the conditions that mercerized pulps would show the most peculiar behaviors in the reaction. The solubilites of pulp, γ-values of undissolved part of pulp and viscose were determined at various reaction times.

The findings on the behaviors of four kinds of pulps are discussed.

128. On alkali solubility curve of pulp. Part 1. Fine structure of cellulose (1). Osakazu NAKAO, Yasuhiko KUROSAWA and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan 13, 591–596, 1957)

In order to investigate the influence of fine structure on the alkali solubility curve of pulp, the hydrolized residues (crystalline region and crystalline region containning mesomorphous region) were prepared from linter pulp, rayon wood pulp and bleached sulfite paper pulp, by dilute acid hydrolysis for 30 minutes and 3 hours respectively, and their alkali solubilities were determined.

The degraded cellulose samples were prepared from linter pulp and rayon pulp by irradiation of ultra violet rays, and their alkali solubility were also determined.

From the results we obtained, the influence of the fine structure and the degree of polymerization of pulp on the alkali solubility curve of pulp are discussed.

Part 2. Fine structure of cellulose (2). Osakazu NAKAO, Saisei MIYAO and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan **13**, 596–600, 1957)

In the previous paper the influence of fine structure of cellulose on the alkali solubility curve of pulp were reported. In the present paper linter pulp and rayon wood pulp were nitrated with two kinds of acid mixtures and then denitrated so that the degree of polymerization of cellulose falls little, and the alkali solubilities and distributions of lateral order were determined on the samples thus obtained.

From the results obtained further discussions are made on the influence of fine structure on the alkali solubility curve of pulp.

Part 3. Influence of outer-layer of cell wall. Osakazu NAKAO, Yoshikazu IWAI and Nobuhiko MIGITA (Journ. Japan Wood Research Soc. **3**, 209–214, 1957)

Alkali solubilities were determined by dissolving pulps which had been immersed in water, frozen and then thawed. The freezing treatment ruptured the outer-layer of cell wall of the cellulose fiber by ice crysta formation, and increased the alkali solubility in 6~16% sodium hydroxide solutions. However, little change was recognized in the degree of polymerization and the moisture regain of cellulose fiber. This fact shows that alkali solubility curve of pulp is influenced by the state of outer-layer of cell wall.

From the results obtained in the present and previous papers, it seems that the state of cohesion of cellulose molecule is the most dominant deciding factor in the shaping of the alkali solubility curve of pulp.

129. The influence of pulping process on the reactivity of pulp by emulsion xanthation method. Motonobu ICHINO, Hideji IMAI, Osakazu NAKAO and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan **13**, 739–742, 1957)

In order to investigate the relation between the conditions of pulping process and the reactivity of pulp (M-W & S-W by Bartunek), five groups of samples (twenty-three kinds of samples) were prepared by controlling cooking time, bleaching time, hydrolysis time and alkali treatment conditions. M-W and S-W were determined on the samples thus obtained and on the eleven commercial pulps.

From the results some discussion were made on the influences of viscosity of unbleached pulp, degradation of cellulose by bleaching and acid hydrolysis, and α-cellulose contained in pulp on the reactivity of pulp (M-W, S-W) are discussed. The values of M-W and S-W of pulp depend not only on the viscosity and the α-cellulose content in pulp, but also on the morphological and the fine structure of pulp.

130. Emulsion xanthation of pulp—influence of fine structure on M-W curve. Osakazu NAKAO, Hiromitsu OHYA and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan **14**, 387–394, 1958)

M-W curve, obtained by varying concentrations of sodium hydroxide solution in emulsion xanthation of pulp, may be influenced by some characteristics of pulp, such as distribution of cellulose chain length, morphological and fine structure, etc. In this paper, the relation between M-W curve and the fine structure is studied.

The curve can be characterized by its position and shape. The concentration of sodium hydroxide solution, in which half of pulp goes into solution, was adopted for expression of the position of the curve, and is symbolized with $[\bar{C}]_o$. To express the shape of the curve, we adopted the parameter (n_o) obtained by the procedure, which was proposed by H. Takenaka for representation of the distribution curves of degree of polymerization of high polymers and was modified by us. In this paper the parameter (n_o) means mainly the slope of the curve.

The reltions between the fine structure of pulp and (n_o) and $[\bar{C}]_o$, are as follows;

(1) The slope (n_o) of M-W curve decreases with increasing the uniformity of lateral order distribution and the amount of low ordered region.

(2) If the average lateral order level $[\bar{C}]_H$ becomes lower, the position of M-W curve tends to move toward the lower sodium hydroxide concentration range, namely $[\bar{C}]_o$ decreases. However the position of M-W curve $[\bar{C}]_o$ is not only controlled by the average lateral order level $[\bar{C}]_H$, but also by the distribution of lateral order, especially the relation between the specific surface area and the lateral order level of pulp.

131. The evaluation of lateral order distribution in native cellulose by the emulsion xanthation method. Osakazu NAKAO, Shun-ichi YAMADA and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan **14**, 395–399, 1958)

To evaluate lateral order distribution in native cellulose, fractional dissolution method by the emulsion xanthation was applied with varying concentrations of sodium hydroxide solution. From some preliminary experiments the following conditions were found to be suitable for the purpose. Fifty ml of sodium hydroxide solution and 1.0 ml of carbon disulfide are added to 0.5 g methanolyzed cellulose sample, and treated for 6 hours at 20~25°C. The undissolved reside was washed with sodium hydroxide solution of same concentration as used in emulsion xanthation.

Lateral order distribution curves obtained by this method agree fairly well with that of the fractional dissolution method proposed by T. YUYUGI and H. MAEDA.

132. On the mechanism of color reaction of lignin. Part 7. The Mäule color reaction (4). Junzo NAKANO, Kimio HIRAKAWA, Atsushi ISHIZU and Nobuhiko MIGITA (Journ. Japan Wood Research Soc. **3**, 194–197, 1957)

In succession to the previous paper, in which we inferred that the ketone group at α-position in the side chain of lignin molecule is responsible for the Mäule color reaction (Journ. Japan wood Research Soc., **3**, 23, 1957), this paper deals with the carbonyl group by the Mäule treatment. The result may be summarized as follows:
(1) Among the isolated lignins from beech wood, the hydrochloric acid lignin

positive in the Mäule reaction shows much increase of the carbonyl group by treatment with potassium permanganate and then hydrochloric acid, whereas the other lignins negative in the Mäule test show a little increase of the carbonyl group by the same treatment. (2) The increase of carbonyl group at the second treatment with hydrochloric acid is larger than that of the first treatment with potassium permanganate. (3) The quinone structure may be formed in the lignin by the Mäule treatment, but this is not concerned with the color reaction. (4) The first treatment with potassium permanganate is indispensable for the Mäule test. Potassium permanganate may possible act as a reagent of oxidative condensation.

On the mechanism of the Mäule color reaction there remain many problems to be solved. Even the most essential point, whether guaiacyl or syringyl nucleus is responsible for the Mäule test, has not been clarfied. From the knowledge obtained from the authors' studies reported in the present and previous papers, the following schema may be given for the reaction taking place in the Mäule test.

$$\text{Hardwood lignin} \xrightarrow{1\%\ KMnO_4} \text{“Oxidized lignin A”} + \begin{matrix}\text{Reduction products from} \\ KMnO_4\ (\text{mainly } MnO_2)\end{matrix}$$

$$\xrightarrow{3\%\ HCl} \text{“Oxidized lignin B”} \xrightarrow{NH_4OH} \text{Colored lignin (purplish red)}$$

That is to say, the hardwood lignin is oxidized into "oxidized lignin A" at the first step by treatment with potassium permanganate, and then oxidized lignin A thus produced is still more oxidized into "oxidized lignin B" at the second step by the reaction between manganese dioxide, a main reduction product from potassium permanganate, and hydrochloric acid. The chlorine produced at the second step reacts with the oxidized lignin, though the chlorination is not necessary to the Mäule color reaction. Thus oxidized and chlorinated lignin gives purplish red color by the action of aqueous ammonia. This coloring material behaves in a manner similar to phenolphthalein against acid and alkali, namely, its acid form gives a light color and its salt gives a purplish red color. The Mäule reaction is characteristic in a point that coloration is due to the ketone group, newly produced by the oxidation at α-position in the side chain of lignin molecule. Aldehyde group and double bond in the side chain are not concerned with the color reaction.

133. Ester groups in various woods. Junzo NAKANO, Atsushi ISHIZU and Nobuhiko MIGITA (Journ. Japan Wood Research Soc. 4, 1–4, 1958)

Four species of softwood, nine species of hardwood, and six species of tropical wood were subjected to boiling under reflux with a normal sodium hydroxide solution. The following compounds in the alkaline extract were analyzed qualitatively by paper chromatographic method and quantitatively by spectrophotometric method after elution from the paper chromatogram: p-hydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid and ferulic acid. Ultraviolet absorption measurements were made also on the alkaline extracts. The results may be summarized as follows:

(1) Though the alkaline extracts of woods give ordinarily the ultraviolet absorption spectra, similar to that of Brauns' native lignin treated with alkali, the alkaline extracts of aspen and sepetir woods whose ester group contents are exceptionally high, give a different spectra. Therefore, amounts of ester groups in wood may be estimated from the ultraviolet absorption spectrum of alkaline extract.

(2) Aspen wood is peculiar in its remarkably higher content of p-hydroxybenzoate group than that of the others.

(3) A small amount of p-hydroxybenzoic acid is obtained from larch heartwood, but not from sapwood. This probably indicates that the distrobution of ester groups is not universal in a cross section of trunk.

134. Manufacture of dissolving sulfate pulp by xylenesulfonate prehydrolysis. Part 1. Xylenesulfonate prehydrolysis. Junzo NAKANO, Saisei MIYAO, Osakazu NAKAO, Chiyoko TAKATSUKA and Nobuhiko MIGITA (Journ. Japanese TAPPI. 12, 224–227, 1958)

The prehydrolysis treatment of wood chips is generally adopted for the manufacture of disolving sulfate pulp, because this treatment selectively removes pentosan from wood. In stead of water, if xylenesulfonate is used in the prehydrolysis, a considerable amount of lignin will be removed with pentosan, and consequently shortening the time of sulfate cooking after prehydrolysis and the improvement of yield and quality of pulp may be expected.

Beech wood meal and beech wood chips were treated with an aqueous solution of xylenesulfonate to find a suitable condition of prehydrolysis, the varying concentration of reagent, pH, cooking temperature and cooking time. As a comparison the prehydrolysis treatment with water were also carried out. From the results of treatments, the following conditions seem to give a good effect: concentration of xylenesulfonate, 25% in weight; pH of xylenesulfonate solution, 6.1; maximum cooking temperature, 160°C; cooking time at maximum temperature, 2 hours; in this case the yield of prehydrolyzed chips was 71.8%, contents of lignin and pentosan in chip were 19.9% and 12.5%, respectively.

Part 2. Sulfate cooking of prehydrolyzed chips. Junzo NAKANO, Saisei MIYAO, Osakazu NAKAO and Nobuhiko MIGITA (Journ. Japanese TAPPI., 12, 227–232, 1958)

Beech wood chips prehydrolyzed with an aqueous solution of xylenesulfonate and water were cooked by sulfate pulping process, and the rates of dissolution of main wood constituents and the properties of pulps thus obtained were compared. The results are as follows:

(1) If a 25% xylenesulfonate solution (pH 6.1) are used, 120 minutes prehydrolysis at 160°C gives a better effect on the succeeding sulfate cooking than 80 minutes and 160 minutes prehydrolysis.

(2) The xylenesulfonate prehydrolysis gives a better effect on the succeeding sulfate cooking than the water prehydrolysis. In the former the rate of delignification is greater and cellulose is less decomposed during sulfate cooking. However the rates of dissolution of pentosan from chips prehydrolyzed by the two methods are nearly equal during sulfate cooking.

(2) The facts, given above, indicates that the xylenesulfonate prehydrolysis tends to shorten the time of sulfate cooking, and gives pulp of high α-cellulose content at higher yield. The distribution of polymerization degrees of cellulose in pulp, prepared by the xylenesulfonate prehydrolysis, is more homogeneous, too.

(4) Though the experiments of bleaching and purification of unbleached pulp have not been carried out, it may be expected that the xylenesulfonate prehydrolysis gives more bleachable pulp than the water prehydrolysis at same pulp yield. By

the xylenesulfonate prehydrolysis, the preparation of pulp where α-cellulose content is not less htan 97% may be also expected without special treatment of purification.

135. Terpenoids. Part 2. Composition of the essential oil from the leaves of "Sugi" (*Cryptomeria japonica* D. Don). Tomoichiro NAKATSUKA, Yoshiyuki HIROSE and Mamoru MATSUDA (Journ. Japan Wood Research Soc. **3**, 206–208, 1957)

The leaves of "Sugi" were steam-distilled to give an essential oil in a yield of 0.12%. It has been found that α-pinene, camphene, dipentene, *p*-cymene, terpinenol-(4), cadinene, α-podocarprene and α-cryptomerene are contained therein.

Part 3. Composition of the essential oil from the wood of *Biota orientalis* Endl. (*Thuja orientalis* L.). Yoshiyuki HIROSE and Tomoichiro NAKATSUKA (ibid. **4**, 26–29, 1958)

The essential oil obtained from the wood of *Biota orientalis* Endl. by steam-distillation was investigated. It has been found that thujic acid, β- and γ-thujaplicins are contained in the acidic portion and widdrene and cedrol in the neutral portion. Widdrene was confirmed to be identical with thujopsene which was isolated from *Thujopsis dolabrata* Sieb. et Zucc. by J. Kawamura.

VIII. Zootechnical and Veterinary Science

Abstract Nos. 136—166

136. Oxygen consumption and lactose synthesis rates of mammary gland slices from lactating guinea-pigs. Motoo NAITO (Journ. Dairy Res. **25** [1], 17–23, 1958)

The shape of the lactation curve in guinea-pigs has been studied. The peak was found to occur between days 2 and 3.

The oxygen consumption and lactose synthesis rates were investigated in mammary gland slices and results expressed on both DNA and DW bases.

Oxygen consumption rates on a DNA basis tended to be low at the onset of lactation and high at the peak. Lactose synthesis on a DW basis tended to decline linearly from the onset of lactation, but on a DNA basis, showed a peak, coinciding with that of the lactation curve.

The significance of these findings has been discussed.

137. Relation between dairy perfermance and mammary glands and their stimulators. Part 2. Estrogen level in the blood of goats treated with estrogen for induced lactation; particularly on the method of biological assay. Hiroshi NAGASAWA, Yoichi SHODA and Motoo NAITO (Jap. Journ. Animal Reprod., **4** [2], 79–82, 1958)

This study has been done in order to clarify the effect of Euvestin (diethylstilbestrol) sol instead of Euvestin oil for the convenience of practical application, in the case of induction of lactation in goats, which has been studied for testing the possibility predicting dairy performance in dairy animals.

The object of this report is to present one modified biological assay method

for measuring small amounts of estrogen in the blood, and note the changes of estrogen level in the blood of goatlings treated with Euvestin sol or oil.

Three Saanen and one Alpine goatlings were divided into two groups. The treatments are as follows; animals of group I were injected intramuscularly with 2.5 mg Euvestin sol twice every 10 days. After 13 days from the 2nd injection, 20 mg Euvestin sol was added two times with 10 days interval. Those of group II were injected subcutaneously with 0.25 mg Euvestin oil daily for 20 days.

The results obtained are as follows.

(1) Our assay method was almost similar to that of *Fujii et al.* (1955), which was one of local vaginal response methods. But in considering the persistency of the effect of a test substance and the simplicity of judging so on, a little modification was done; 48, 60 and 72 hours after the 1st insertion of the test substance to the vagina, smears were tested and the points of 3, 2, 1 and 0 were given to the signs of (++), (+), (±) and (−) respectively. Then, the index of the response was determined as the average of the scores of mice for three periods calculated.

According to this method, 2.44 was equal to 1 Vaginal Unit of *Allen-Doisy* Method.

(2) The changes of the level of estrogen in the blood of Euvestin treated goatlings were very fast. The estrogen level of the animals of group I was maximum on the 6th day after the 1st injection and fell down rapidly even after the 2nd injection. Those of group II was maximum on the 15th day after the 1st injection and afterwards fell down in spite of the continuous injection for 20 days.

Further studies may be necessary for clarifying these phenomena mentioned above.

138. Establishment of mice strains with special reference to mammary growth response. Part 1. Comparison of several measuring methods of mammary duct areas in immature mice treated with estrogen. Jiro NAGAI and Junzo YAMADA (Endocrinologia Japonica 4 [3], 179–183, 1957)

The study was carried out to obtain simple and considerably accurate method for measuring mamary duct areas in mice treated with estrogen. Normal and gonadectomized Kasukabe mice of both sexes were injected on five occasions subcutaneously with total dose of 12.5 I.U. of synthetic estrogen from the time they were 20 days old. On the 35th day, the mice were killed and the third thoracic mammary glands were prepared by whole mount method. Preparations were enlarged on section paper and such four methods as A) Index of mamary duct area method, B) Planimeter method, C) Calipers method-1, and D) Calipers method-2 were used to measure the mammary duct areas.

Results obtained are as follows.

1. Planimeter method is comparatively reliable among B), C) and D) method.
2. There are close associations between mammary duct areas measured by A) and B) method. Correlation coefficients are 0.85 in female, 0.95 in castrated female, 0.94 in male and 0.77 in castrated male.
3. The differences of mammary duct areas between normal and gonadectomized group are not sifinificant in each sex but the difference between sexes is significant

statistically. When normal and gonadectomized group's data in each sex are combined, means with fiducial limits by B) method are 175.3±4.6 in female and 179.8±8.2 in male when the mammary duct areas measured by A) method in each sex are placed as standards respectively.

Part 2. Relationship between mammary growth response and body weight in immature female mice treated with estrogen. Jiro NAGAI, Motokazu YOSHIDA and Motoo NAITO (Jap. Journ. Zootech. Sci. 28 [5], 287–290, 1957)

Relations between mammary growth response to constant dosage of estrogen and body weight in mice and their heritabilities were investigated.

The number of mice used was 259. They were born from 52 dams which were on the 3rd generation of full-sib mating system. They were injected five times subcutaneously with a total dose of 12.5 I.U. of synthetic estrogen every third day from 20 days of age. On the 35th day they were killed after their body weights (W) were weighed. Mammary growth responses (M: mammary duct areas in mm^2) and corrected mammary growth responses for average body weight were obtained.

Heritabilities on the 3rd generation were estimated as 0.32, 0.36 and 0.25 in M. W. and corrected mammary growth response respectively, using the method of variance analysis and genetic correlation between parent and offspring.

Relationship between two characters (rMW) was 0.62 phenotypically, and 0.86 genetically.

In conclusion, mammary growth response was a heritable character as well as body weight, and a close relationship in a genetical sense was present between body weight and mammary growth response.

Part 3. Changes of variation in mammary growth response of mice inbred up-to the 6th generation of full-sib mating. Jiro NAGAI, Motokazu YOSHIDA and Motoo NAITO (Jap. Journ. Breed. 7 [4], 260–263, 1957)

Breeding experiments were carried out for the purpose of ascertaining the possibility of establishing inbred mice strains with less variabilities concernng the mammary growth response which is considered to be a character belonging to "properties of the organism as a whole".

Brother-sister mating system was adopted and selections for less variabilities on the basis of mammary growth response of progenies were carried out on random mating stage in the 2nd generation. The changes of mean and variability of mammary growth response, body weight and corrected mammary growth response for body weight were observed up-to the 6th generation.

Results obtained are as follows:

1) Means of three characters increased gradually.

2) There were no definite tendency for changes concerning gross variances of three characters but squard coefficients of variation decreased gradually.

3) Variances within families compared of brother and sister did not change in definite tendency in each sex. However, squared coefficients of variation within family did decrease gradually in the course of the generation in each sex.

Inbreeding, selection for less variability of mammary growth response and

uncontrolled environmental factor will play important roles while essential factor is not certain for these changes of variability.

139. Electromyographic studies on the function of the skeletal muscles. Part 5. Functional change of the skeletal muscles accompanied by the development. Noriji TABA and Hiroaki SHISHIDO (Jap. Journ. Vet. Sci. **19** [3], 95–99, 1957)

This experiment performed on two brood puppies was designed to investigate the functional change of skeletal muscles accompanying development. Electromyographic recordings of a single neuromuscular unit (NMU) were taken from the gastrocnemius, the quadriceps femoris and the supra spinam of each subject, in the standing posture, once a month from the age of 2 months to the age of 8 months. The discharge intervals of a NMU were measured and analysed according to the formal method. Each discharge-interval-time-series was divided into the two components: the slow undulation and the irregular fluctuation. Taking into consideration the discharge pattern, these components were discussed.

Results are as follows:

1) In the early period of the development, the unusual pattern of discharge is often observed, but in the late period this pattern is scarcely observed.

2) The amplitude of the slow undulation decreases gradually according to the progress of the age, and furthermore, the process of the slow undulation gets stable and rythmical. From these facts, it is clear that the muscle activity to maintain the standing posture is stabilized as the age increases.

3) Compared to the $\bar{\tau}$-S curve at the age of 2 months, the $\bar{\tau}$-S curve at 4 months ($\bar{\tau}$: the average of the discharge intervals, S: the standard deviation of the series of the irregular fluctuation) is displaced to the left side in all muscles, but on the contrary, the $\bar{\tau}$-S curves at 6 and 8 months are displaced to the right side. It is presumed that the displacement to the left side, shown in the early period, results from the development of the nervous system, especially the motor area of the cerebral cortex, and the displacement to the right side, shown in the late period, results from the training of the muscle.

4) The same change is brought about by the development in every muscle, but the grade of change is different due to the kind of muscle.

Part 6. Characters of the discharge-interval-time-series of a single NMU. (1) The $\bar{\tau}$-S curves of the skeletal muscles of animals. Shin-ichi NOMURA, Hiroaki SHISHIDO and Yōko MASUMURA (Jap. Journ. Vet. Sci. **20** [1], 13–18, 1958)

The correlation between the average $\bar{\tau}$ and the standard deviation S of the discharge-interval-time-series of a single NMU has been discussed statistically, using domestic fowls, rabbits, goats and dogs under the conditions of standing on various inclined or moving boards.

The $\bar{\tau}$-S curve of the animals with poorly developed pyramidal tract, that is, domestic fowls, rabbits and goats, is a simple straight line, in which the horizontal part is indistinguishable from the ascending part. However, dogs having well developed pyramidal pathway have the same type of $\bar{\tau}$-S curve as that of the human skeletal muscles. It might be supposed that the former maintains a standing posture in any condition by the action of the subcortical nervous system, evoked

by the afferent impulses from the receptors of the joint, tendon, muscle and other parts of the body. On the contrary, in the latter the inexpedient conditions may activate the cortical motor area to prevent the collapse of a stable stance and bring forth the kinetic function of the neuromuscular system.

The principles governing the $\bar{\tau}$-S curve of the animals with the highly developed pyramidal tract, i.e., dog and cat etc., may be considered the same type as those of the human skeletal muscles.

Part 6. Characters of the discharge-interval-time-series of a single NMU. (2) Statistical analysis of discharge-interval-time-series. Shin-ichi NOMURA, Hiroaki SHISHIDO and Yōko MASUMURA (Jap. Journ. Vet. Sci. **20** [2], 59–65, 1958)

From the statistical standpoint, a discharge-interval-time-series of single NMU consists of several kinds of fluctuations with different periods. The repetition of the arithmetical moving average method can divide the original series into those of two types. They are the slow undulation, the series of moving averages and the irregular fluctuation, the series of deviations from the moving averages. Applying WHITTAKER's periodgram analysis to the series which remains after the elimination of the spiky fluctuation from the irreglar fluctuation, the harmonic wave component (or the H-type fluctuation) is extracted. And, on the contrary, the spiky component (or the R-type fluctuation) remains after the elimination of the harmonic wave by means of the moving average method.

The same time-series discussed in the preceeding report were analysed again by the above-mentioned methods and the peculiarities of the subdivided elementary fluctuations were asserted.

1) The average amplitude A of the slow undulation widens in proportion to the mean value $\bar{\tau}$ of the original time-series in all of the animals observed. In the dog, however, the A has a settled value until the $\bar{\tau}$ exceeds 70∼80 ms and thus the $\bar{\tau}$-A curve has a horizontal part.

2) The H-type fluctuation generally consist of two harmonic wave components of different periods P and therefore, the $\bar{\tau}$-P points distribute along two different regression curves. The one is called the C-curve and it is supposed to represent the period of the sway of activity in the motor area of the cerebral cortex. The other is called the S-curve which represents the periodic fluctuation in the sub-cortical nervous system. The period of each grows with the increase of the $\bar{\tau}$, but has a certain maximal value. It is assumed that the increase of the cortex activity (or corticalization) in the control of muscular contraction brings an increase of inclination of the C-curve and the reinforcement of the lower motor neuron system (or spinalization) results in the lowering of the maximal values of both curves. Every conditioning causes the spinalization in the rabbit and goat, the lifting of the maximal values in the C- and S-curve in the dog.

3) The period and decrement of the R-type fluctuation calculated by means of the correlogram analysis show the relationship between the adjoining motor impulses sent out from the spinal cord. In rabbits and goats no change is manifested in any of the various standing postures. But in the dog the seesaw movement gives rise not only to the prolongation of the period, but also to decrease regularity in the adjacent discharge intervals in the long muscles passing over several joints.

Therefore, it is supposed that the rabbit and goat always maintain their erect position by the activation of the lower motor neuron system, but in the dog the cortical nervous system gives rise to phasic contraction of the essential muscles for the prevention of disturbance of the standing posture.

Part 6. Characters of the discharge-interval-time-series of a single NMU. (3) The $\bar{\tau}$-S curves of the skeletal muscle of in the dog. Shin-ichi NOMURA, Hiroaki SHISHIDO and Yoko MASUMURA (Jap. Journ. Vet. Sci. **20** [3], 99–103, 1958)

The results described in the preceeding reports, that the $\bar{\tau}$-S curve from the original discharge-interval-time-series of the skeletal muscles in the dog has a horizontal part, suggests that the curve may be divided into the K- and T-curve as in man. The K-curve represents the characteristic of the K-NMU or the motor units being involved in phasic muscular contraction, and the T-curve represents the peculiarity of T-NMU exerting tonic contraction.

Using two trained dogs, a number of discharge-interval-time-series of different mean values were obtained under the various standing postures.

It seems that the $\bar{\tau}$-S points of the muscles investigated are able to be summarized into two large groups; one is that of the K-NMU and the other that of the T-NMU. By further strict analysing, each of the groups may be divided into three smaller groups; the 1st group is obtained from the axial muscles and is partial to the extreme left side; the second is that of the muscles in the extremity girdles and is located between the 1st and the 3rd group; the 3rd is that of the limb muscles and stands at the extreme right side with the longest horizontal part and the slowly ascending part.

While the NMU of the skeletal muscles in the dog, as stated above, may be divided into the kinetic and tonic NMU as in man, the ambiguity of this assumption should be remembered, because of the fact that the $\bar{\tau}$-S points distributing along the K- or T-curve in the present experiments have not been always obtained from different time-series.

140. Studies on artificial insemination of rat. Part 3. On the rhythem of vaginal smear and the time of ovulation in the rats kept under the condition of artificially reversed day and night. Shōzō OGAWA and Yoshisuke SUZUKI (Jap. Journ. Animal Reprod. 3 [2], 75–77, 1957)

Part 4. On the optimum period of insemination in rats kept under artificially reversed day and night. Shōzō OGAWA and Yoshisuke SUZUKI (Jap. Journ. Animal Reprod. 3 [2], 78–80, 1957)

141. On the delayed implantation in the lacting pregnant rat. Part 1. The effect of estrogen. Kōzi YOSHINAGA and Tōshirō HOSHI (Jap. Journ. Animal Reprod. 3 [2], 93, 1958)

In rodents the implantation of fertilized ova does not take place at the normal time but daloys for some days if the female suckles such a large litter as five or more.

Convinced of this delayed implantation in rat, w have tried to normalize it by estrogen administration.

Various doses of estrone (0.1, 0.3, 0.5 and 1.0 μg) were injected subcutaneously on the 5th day of pregnancy to the females suckling 6 pups. Examination for implantation sites on the 9th day of pregnancy showed that 0.1 μg of estrone did not induce normal implantation but delayed it in the three females; on the other hand, larger doses of estrone induced normal implantation, that is, 0.3 μg induced it in 2 of 7 females, 0.5 μg 5 of 7 and 1.0 μg 6 of 6 respectively.

These results show that an estrone dose of more than 0.3 μg could normalize the delayed implantation in the lactating rat. But if the suckling litter is less than e.g. 2, the implantation does not delay. This fact suggests that the delay of implantation depends upon the amount of milk secretion or the suckling stimuli to the teats of the mother. So, in all females, the increase in body weight of each litter between 2nd and 7th day of pregnancy, was tested to know if there were any difference between the two groups: one of these groups, consisting of 10 females showed delayed implantation, the other of 13 females showed normal implantation. There was no significant difference in increase in body weight of each litter between these two groups.

These findings, therefore, make it clear that the estrone was able to induce the normal implantation in suckling rats.

142. Some effects of ovarian hormones on the fluctuation of ovulating hormone content of rat hypophysis. Part 1. Ovulation blockade by nembutal sedation in our wistar strain rat. Teiichi ETO, Hiroshi TOYODA and Toshiro HOSHI (Jap. Journ. Animal Reprod. 3, 113-116, 1958)

In rat, which is a spontaneously ovulating animal, the studies by Everett et al. demonstrated that the critical period at which the release of LH for ovulation is provoked with the neurogenic stimulus is between 2~4 P.M. on the day of proestrus, and estrogen or progestrone affects on the release of LH through the nervous system. However, their method involved the confusion of the actions of endogenous and exogenous overian steroids.

The authors' experiments were carried out to obtain more detail figures concerning the effect of overian steroids on the neurogenic timing factor by the elimination of the confusion.

In this report, the preliminary runs were performed by the tracing the Everett's studies to decide the regularity of cycles and the relationship between activation of the rat hypophysis by neurogenic stimulation and time of day in our Wistar strain rat.

The experiments consist of two series, one is controlled the lighting by a time switch, the lights being on for 14 hours each day, the other is not controlled.

The regularity of vaginal smear cycles in our strain after two successive observed cycles of 4-days in sequence is such that under the lighting controlled condition there is a probability of 97% that the next cycle will also be of 4 days length and the probability is slightly decreased under the non-controlled condition.

The rat which had a history of at least two 4-day cycles in sequence was immediately injected intraperitonialy with nembutal (40 mg./kg. body weight) at the 2-4 P.M. of the day of proestrus, the ovulations in most of those animals were prevented without concerning the control of the lighting.

The results confirmed closely Everett's reports.

143. A new biological test of androgen, based upon the epididymal growth. Sumio SHIMA and Yoshisuke SUZUKI (Jap. Journ. Animal Reprod. 3, 89, 1958)

One of the authors, SUZUKI (1955), has found that in the unilateral orchidectomized immature rat the epididymis of the non-castrated side grows more rapidly than the other.

On the basis of this finding, a new biological test of androgen was studied. The infant male rat was castrated and implanted with a cylindrical vinyl sponge pellet containing the steroid hormone into the unilateral scrotum, and after 4 days the animal was sacrificed and the genital organs were weighed. The asymmetry of the epididymal growth, expressed with the ratio of the epididymal weight of the implanted side to the other, was shown as the index of local action of the action, and the growth of seminal vesicles and prostate glands expressed with mg. per 100 g. of the body weight as the index of systemic action.

On testosterone: The local action of androgen on epididymis was relatively peculiar in testosterone and clear asymmetric growth was obtained in the range of 25-100 μg. With a dose over 100 μg. the systemic action appeared on seminal vesicles and prostates, and asymmetric growth was masked.

On estrone: The local action of estrone on epididymis was obscure. However, its strong systemic action was obtained with a dose over 50 μg.

On androsterone: Androsterone did not bring about any asymmetric growth at any dose. The systemic action of androsterone on seminal vesicles was not clear in comparison with testosterone, but on the prostate, obvious effect was obtained with a dose over 250 μg.

On testosterone—estrone: It was observed that testosterone and estrone had a tendency to act antagonistically to each other when they were given simultaneously.

144. Further studies on the direct transportation on testis hormone to the targets located close to the testicle. Yoshisuke SUZUKI and Sumio SHIMA (Proc. 3rd Intern. Congress Animal Peprod. Cambridg, 1956)

In the tunica vaginalis communis of male goat, a small amount of yellowish transparent fluid is always discovered.

According to the anatomical study, this fluid is not the abdominal serosity flowing into the scrotal cavity, but exduation from the adjacent tissues or organs of the testis.

The androgenic activity for stimulation of the epididymal growth was demonstrated in the ether extract of this scrotal fluid, by the method of the local application to the castrated infant male rat.

The androgenic activity of this fluid is estimated to be about 10 times greater than that of serum.

Therefore, it may be reasonably concluded that the concept, in which the testicular androgen is transported to the targets located close to the testis, not only via the systemic blood circulation but also via some direct route, may be applicable to the goat as well as the rat, even in the mature stage of life.

145. Acethlcholine in the stretched wall of intestine. Miyoshi IKEDA, Toyoaki HAYAMA, Nobuyuki CHUJYO and Akio HOSHI (Amer. Jour. Physiol. **192**, 27-29, 1958)

In this study, the acetylcholine content of the guinea pig ileum and the effect on it of the circumferential stretching of the intestinal wall were studied, and the preliminary study showed that there is a loss of potassium from the stretched tissue. The total acetylcholine content decreased immediately after stretching, which effect was inhibited by eserine. Release of acetylcholine and its enzymatic destruction may be responsible for the decrease of acetylcholine. Changes in potassium content of the enviromental fluid of the intestine were also studied. The results suggest that the circumferential stretching augments the loss of potassium from the tissue, and this effect of stretching was enhanced by eserine.

146. Significance of plexus on the appearance of peristalysis in the isolated intestine. Iwao YANAGIYA and Yoshio OHKUBO (Journ. Physiol. Soc. Japan **20** [6], 460–461, 1958)

The physiological basis of the peristaltic reflex in the isolated intestine is still not fully understood. In this paper, the polar response of contraction to the local distension stimulus by means of the balloon or the Tyrode's fluid infusing (a new method) is described in detail. By means of the balloon, the local distension stimulus elicited the initial contraction orad (polar response). Through the application of ganglion blocking drugs, hexamethonium, T.E.A.B. and cocaine, contractions near the stimulated region disappear.

If polar response depends only upon the myentric reflex, and spontaneous contraction is myogénic in origin (Bozler, 1949), the contractions may be produced at the maximum distension part even when the plexus has been paralysed. By means of the balloon, however, we have not been able to observe this contraction under these conditions. Therefore, in order to stimulate the local part of the intestine by means of Tyrode's fluid, a piece of intestine (3-5 cm. as in Trendelenburg's method) is inserted into a glass tube swollen globularly (approximately 1.0 cm. diameter) at the central part. Increasing the intraintestinal pressure, the intestine is distended locally at the part of the glass globular swelling. In these experiments, local distention stimulus has also elicited the initial contraction at the oral region, and when the plexus has been paralysed by application of ganglion blocking drugs, a great number of initial contractions appeared at the middle region of the glass swelling and propagated to the aboral direction.

Peristalsis, by Trendelenburg's method, presented no regular contractions under conditions in which the plexus had been paralysed, and only restricted contractions appeared in several parts of the intestinal preparation at the same time. But by increasing the intraintestinal pressure, additionally, peristalsis presented regular contractions again under the same conditions.

Thus, we assumed that the polar character of the myenteric reflex explains why the peristaltic waves of the intestine generally travel only in an aboral direction. The reflex itself, however, does not strictly control the conduction mechanism of the peristaltic waves. The mechanism of occurrence and conduction of peristalsis and anti-peristalsis is discussed in relation to the myenteric reflex and the propelled contents of the intestine.

147. Significance of longitudinal and circular muscle layer on the appearance of peristalsis. Iwao YANAGIYA, Yoshio OHKUBO and Masakazu SHIMADA (Journ. Physiol. Soc.

Japan **20** [6], 467-468, 1958)

It has been demonstrated in our previous communication (Yanagiya & Ohkubo, 1955) that the distension of the intestinal wall is essential for the occurrence of peristalsis. In this experiment, we have studied the contractions of both the longitudinal and circular muscles of the isolated guinea-pig intestine, caused by raising the pressure in the lumen. When the lumen of the isolated intestine is filled with Tyrode fluid, a peristaltic reflex is set up in which a conductive contraction of the longitudinal muscle is followed by a contraction of the circular muscle, expelling the fluid from the lumen (Trendelenburg, 1917). The purpose of this communication is to show that a partial analysis of the mechanism of the contraction of the longitudinal and circular muscle has been carried out.

In order to record the contraction of the longitudinal muscle without the influence of the contraction of the circular muscle, the intestinal strip is covered with a glass tube (a modification of Trendelengurg's method), and prevented from distending under rising pressure. A short and non-rhythmical contraction of the longitudinal muscle arises with more than 20 mm (Throde fluid) intra-luminal pressure, and increases under increasing pressure, but decreases rather more than 60 mm when preceded with a short initial lengthening of the longitudinal muscle. This longitudinal muscle contraction does not usually continue until the lumen is emptied again.

Acetylcholine and ergotamine increases this contraction, on the other hand atropine and adrenaline decreases it; therefore, it is assummed that the contraction is an active reaction to the intraintestinal pressure.

Fixing both ends of the intestinal strip to avoid the longitudinal muscle contraction as in the Trendelenburg's method, rhythmic contraction waves of the circular muscle expelled the fluid from the lumen, showing that peristalsis existed. The effects of acetylcholine, atropine, adrenaline and ergotamine on this circular muscle contractions are similar to those produced by these drugs on the peristalsis by Trendelenburg's method.

It was found that the essential phenomenon for or origination of the peristalsis is not the longitudinal muscle contraction but the distension of the circular muscle layer.

148. Effect of the viscosity of intestinal contents on the progressing rate of peristalsis. Iwao YANAGIYA and Yoshio OHKUBO (Journ. Physiol. Soc. Japan **20** [6], 474-475, 1958)

In our previous study (Yanagiya & Ohkubo, 1957) the mechanism of the occurrence and conduction of peristalsis and anti-peristalsis in relation to the myenteric reflex and the propelled contents of the intestine has been discussed.

In this experiment, performed with the preparation of the excised small intestine of the guinea pig, the relation between the propagating rate of peristalsis and the viscosity of intestinal contents has been observed as follows.

A long piece of excised intestine (about 30-40 cm) is laid in Tyrode's solution, after being connected with two levers at two positions at approximately 10 cm intervals. The peristaltic waves, originated by infusing the examined fluids into the lumen at the oral end, are recorded on the kymograph through two levers. With

these recorded curves the propagating rate of peristalsis has been determined by the distance of the two contraction. The following fluids, showing different degrees of viscosity, have been infused into the lumen as experimental samples; water (specific viscosity=1 at 38°C), NaCl 0.5% (s.v.=1), NaCl 2% (s.v.=1), glucose 2.5% s.v.=1), glucose 10% (s.v.=1), Tyrode's solution (s.v.=1), agar 1g/dl (s.v.=3.1), millet-jelly (millet-jelly: water=2:1) (s.v.=3.5), arabia gum 2g/dl (s.v.=5.2), paraffin liquid (s.v.=6.0), starch 2g/dl in paste (s.v.=7.0), starch 4g/dl in paste (s.v.=97.0).

These fluids had no influences on the intestinal motility in application for the mucosa, and the osmotic pressures shown by these fluids is less than that of NaCl 2% solution which also has no influences. Results obtained show that the fluids showing a low viscosity (s.v.=1), caused a fast progressing perstalsis (7.0-11.5 cm/sec, mostly about 8 cm/sec). On the other hand the fluids showing a higher viscosity (more than 3.1), caused slower peristalsis which were similar in their physiological progressing rate in vivo (3.5-7.5 cm/sec, mostly 4 cm/sec).

149. Factors necessary for *E. tenella* infection of the chicken. Part 5. Organ specificity of *E. tenella* infection. Miyoshi Ikeda (Jap. Journ. Vet. Sci. **19**, 105–113, 1957)

The present observations were carried out in order to get further evidence regarding organ specificity of infection by E. tenella. The following results were obtained.

(1) According to the preliminary observations on the passage of ingesta through the tract with markers (charcoal or Lycopodium used), it was found that some of the feed first ingested reached the entrance of the ceca within about 1 hour after feeding and one part could be slightly detected in the cecal lumen within 2 hours and more markedly about 3 hours later. It required about 2-3 days for the disappearance of the marker, which had entered into the ceca, from the cecal contents or droppings up to the undetectable level.

(2) As the mechanism for the evacuation of cecal contents is unknown, observations were carried out on chickens under the normal condition in the laboratory with natural light and the report by Herrick et al. (1947) was confirmed. The following results were obtained: a) The average daily droppings of cecal passage by chickens were 3-4, but no cecal droppings during the night from the sleeping time (7-8 p.m.) to natural awakening the next morning (4-5 a.m.). b) Two peaks, morning (5-7 a.m.) and afternoon (3-7 p.m.), appeared in the cecal evacuations without the effects due to the administration of food and water. The morning peak was more abrupt, but the afternoon peak was larger and more sluggish. c) On the contrary to the cecal dropping, the intestines was always evacuated throughout the day and night, indicating no definite peak of passage.

Further attempts were carried out to astertain the possible effects of natural light and the body movement of the chicken on the passage of both cecal and intestinal droppings. In the case of the chickens deprived of natural light, by keeping them in a dark room, no cecal droppings passed during the day and night. On the other hand, in the case of the chickens kept in a cage with normal natural light but allowing only the head and neck to be free for the purpose of suppressing the movement of the whole body, with such a posture as that when being asleep, the intestinal droppings, but not the cecal, passed.

From the data obtained, it seems that the passage of the cecal droppings is closely related to the movement of the chicken.

(3) In order to test further the effects of the cecal contents on infection, a distal end of one side of two cecal pouches was sected and its cecal segment was surgically transplanted to different locations from the upper duodenum to the lower intestine, thus causing food to pass through the translocated ceca, while the other side was left as normally for the control.

Following the introduction of sporulated oocysts into the chickens so operated upon, no infection in the united ceca occured in the case of the union to the duodenum, with the exception of the union to the distal part of the loop of the duodenum, but in this case when the united ceca to the lower intestine became infected, it was less severely infected than in the case of another ceca lying in the normal position.

(4) Following the administration of sporulated oocysts to the chickens from which two cecal pouches were surgically removed, the chickens became infected, resulting in 3 deaths out of 5, and at autopsy, showing the characteristic hemorrhage and lesions produced at the lower end of the intestine and rectum adjacent to the entrance of the ceca into the intestine.

From these results it was suggested that the organ specificity of the infection of E. tenella in the chicken might be, essentially, rather dependent upon the close affinity that exists between the host cell and the parasite, than to the characeristic conents of the ceca and their retention here, though the latter factors do not necessarily mean that they play no role in the infection as discussed before.

However, it seemed probably that the lower part of the intestine and rectum, seemingly less suitable for the growth of the coccidia, significantly changed in order to become suited for its development under certain conditions. The mechanism for the specificity and the change of the parasitic distribution remains unknown and awaits a solution by further experimental evidence.

150. Studies on Leptospira. Part 1. Laboratory Technics for Serological Investigation. Shutaro YAMAMOTO (Proc. Japan Acad. 33, 564, 1957)

The serological classification of strains of pathogenic leptospires is not only a subject of academic interest but also a matter of practical diagnosis and serotherapy of, and vaccination against, leptospirosis. In the early studies on leptospirosis in Japan, there were used Oba's agglutination tests in vitro, and Pfeiffer's tests on guinea pigs injected with 0.5 ml of undiluted immune serum and 1 ml of guinea pig liver emulsion containing leptospires. Recently the author modified Schüffner and Mochtar's agglutination-lysis technic, which has been playing an important role in the investigation in Japan. He also adopted a new notation system to express the results of the former test. These contributions are believed to facilitate the serological studies on leptospires almost as well as on other microorganisms. These modifications are playing an important role in the recent advancement of research in leptospirosis in Japan.

Part 2. Serological Classification of Leptospira Strains found in Man and Animals. Shutaro YAMAMOTO (Proc. Japan Acad. 34, 56, 1958)

The causal organism of Weil's disease, *Spirochaeta ictero-haemorrhagiae*, was

isolated for the first time in 1915 by Inada and Ido in Japan. Then, in 1918, the specific cause of Nanuka-yami, or seven day fever, *Spirochaeta hebdomadis*, was discovered by Ido and his co-workers, and in 1925 that of Akiyami, or autumn fever, by Inada's fellow workers, Koshina, Shiozawa, and Kitayama, in Japan. These authors were pioneers in studies on leptospirosis in the world. In their studies they advocated the serological differentiation and typing of Leptospira, which recently has become one of the most important problems on Leptospira. This article deals with the serological classification of 64 Japanese and foreign strains of leptospires. Six Japanese and 58 foreign strains were used. These pathogenic leptospiral strains were serologically compared with the technics which had been discussed in the author's previous paper. From serological relations, the author tentatively established 12 groups among the 64 strains. The serological position of nine strains from Belgian Congo was determined.

151. Pulmonary acariasis in a monkey (*Mac. nemestrinus*). Shutaro YAMAMOTO, Kiichi ISHIDA and Akio SATO (Jap. Journ. Vet. Sci. **19** Suppl. 49, 1957)

The first case of pulmonary acariasis in monkey was reported by DE HAAN and G. GRIJNS, in 1901. There are 5 species of Genus *Pneumonyssus* known to parasitize in the respiratory organs of monkey. But *Pn. simicola*, discovered by DE HAAN in 1901, has been the only species that causes pulmonary lesions.

In July 1956, very small mites were found in the pulmonary lesions occuring in the monkey (*Mac. nemestrinus*) imported from India about a week before and brought for postmortem diagnosis to our laboratory.

The lesions appeared as greyish white poppy seed size nodules distributed mostly beneath the pulmonary pleura. One to several greyish white or yellow mites were contained in each lesion. 7 males, 49 females and 33 larvae were obtained but no nymphs. It was noted that the mumber of males was by far the smaller than that of females or larvae. According to their morphological characteristics they were identified as *Pn. simicola* described by OUDEMANS.

Microscopically, pulmonary lesions were the cavities surrounded by granulation tissues with infiltration of small round cells and a few esosinophiles. Most part of cavity wall had a layer of squamous or cuboidal epithelium supported with a thin layer of hyalinized connective tissue. Excrements of mites (Guanine) deposited in and outside of the cavity. The same change was found around the normally lined bronchi. The mites were found in the cysts as well as in the apparently normal bronchi.

152. Experimental study on radiation injury.—Hematological and pathological observation in chicken. Yoshio OKUBO, Shutaro YAMAMOTO, Miyoshi IKEDA, Kiichi ISHIDA, Akio SATO and Osamu MATSUOKA (Jap. Journ. Vet. Sci. **19** Suppl. 76, 1957)

In order to study the hematological and pathological changes in chicken with injection of radioactive materials, 2 $\mu c/g$, 5 $\mu c/g$ and 10 $\mu c/g$ of P^{32} were injected into male chickens (300g in body weight) intraperitoneally. At an interval of 2-3 days the peripheral blood counts of the chicken of each group were performed by WISEMAN's method during a period of 35 days after injection.

The results were as follows:

1) All the chickens injected with 5 $\mu c/g$ and 10 $\mu c/g$ of P^{32} died within 15 days after injection. Their erythrocyte counts decreased suddenly just before death and most were below 600,000 per c.mm. Decrease of lymphocytes was rapid during the first 2 or 3 days. Granulocytes decreased gradually and at last completely disappeared from the peripheral blood.

The important pathological findings of these groups were (1) Systemic hemorrhage, (2) distinct atrophy of spleen and thymus and (3) red bone marrow becomes fatty or gelatinous marrow.

2) None of the chicken injected with 2 $\mu c/g$ of P^{32} died within our observation period. Erythrocytes continued to decrease 10 days after injection, reaching below 2,000,000 per c. mm. and then recovery had taken place steadily. It recovered to normal 35 days after injection. Lymphocytes decreased also within the first 24 hours and then the recovery began 7-9 days after injection. Granulocytes continued to decrease more gradually during 15 days after injection and the recovery was more slower than that of lymphocytes.

153. Isolation of *Crypthcoccus neoformans* from pulmonary granuloma of a cat and from pigeon droppings. Shutaro YAMAMOTO, Kiichi ISHIDA and Akio SATO (Jap. Journ. Vet. Sci. **19**, 179–191, 1957)

There has been an increase in the incidences of human cryptococcosis in various countries. Several species of animals are also known to have been infected with this fungus.

In Japan, several proved cases of cryptococcosis in man were reported recently, but no distinct cases have been reported in the case of animals.

In March 1957, a strain of *Cryptococcus neoformans* was isolated from the pulmonary granuloma occuring in a cat, brought for autopsy to our laboratory. The cat had suffered from a stubborn diarrhea and received various antibiotics without success. At autopsy, no significant changes were noted except for a yellowish white, round nodule about 5 mm in diameter, at the margin of the right lower pulmonary lobe. The cut surface of the lesion revealed caseation necrosis. Smear preparation of this lesion revealed numerous yeast-like, spherical bodies, each surrounded by a wide hale in the necrotic substances. The infection with a yeast-like fungus was at once suspected and cultures were made from the granuloma on Sabouraud-ager-slants. After two days of incubation at 37°C, a pure culture of round, slightly elevated, glistening white, pasty colonies was obtained. Under the microscope they presented themselves as spherical budding yeasts with distinct cell walls. This strain was identified as *Cryptococcus neoformans* by the system for the classification of the yeasts by LODDER and KREGER-VAN-RIJ and BENHAM's criteria.

Cryptococcus neoformans was isolated for the first time from a saprophytic source, viz. from the fermenting peach, by SANFELICE, in 1894. But it had not yet been known that a certain material was constantly, or very frequently, associated with this fungus, until C. W. EMMONS' report on the isolation of *Cryptococcus neoformancs* from pigeon droppings, in 1955. Special interest has been aroused in our minds as to whether this might also be the case in Japan and 24 specimens of pigepigeon droppings were collected from 5 places in the Tokyo City area during the period of spring to early summer in 1957. These were examined by Emmons'

method for the presence of *Cryptococcus neoformans*. 19 strains of typical *Cryptococcus neoformans* were isolated from the 24 specimens.

154. Studies on the infection of chick embryos with bovine and avian tubercle bacilli. Shutaro YAMAMOTO, Kosaku FUJIWARA, Katsumoto UEDA and Shoichi FUKUDA (Jap. Journ. Exp. Med. **27**, 417–423, 1957)

Analyses of infection in embryonic life are difficult because of continuing evolution. It seems, however, to be interesting to observe infection of emryos which is considered to be inactive for immunity or allergy in contrast with adults. The experiments reported here were attempted with this viewpoint which might throw light upon the possibility to reveal some phenomena behind the complicated processes of infection in adults.

Chick embryos and bovine and avian type tubercle bacilli were considered to be convenient for these experiments and therefore utilized.

An attempt to infect chick embryos with tubercle bacilli had been made by many authors who inoculated the bacilli into the chorio-allantoic membrane or yolk sack. In 1947, LEE and STAVITSKY injected human tubercle bacilli into chick embryos intravenously for the purpose of a simplified screening of anti-tuberculous agents. The same route of injection was utilized in the experiments described in this paper, expecting direct effects of inocula to embryonic tissues.

The results were as follows;

1. Bovine type tubercle bacilli, strain RO, produced severe lesions and detered the growth of chick embryos more than a virulent avian type strain, Flamingo, when the organisms were infected intravenously into ten to twelve days' old embryos.
2. The disease of embryos, infected during embryonic life, was not so different in its course after hatching from those infected postnatally.
3. It is likely that the contradictory host-parasite relationship in chick embryos is due to the difference of the site which is stimulated by each type of organisms or to the unstable condition of the embryonic tissues.

155. Studies on the intestinal flora of chickens. Part 1. Cultural methods. Yuichi OCHI and Tomotari MITSUOKA (Jap. Journ. Vet. Sci. **20**, 7–12, 1958)

Recently, nutritional failures in livestock and diseases of the alimentary tract by unknown causes, e. g., infectious diarrhoea, have become one of the most important problems in our country. These problems cannot be solved without studying the relationships between the microflora of the alimentary tract and the host. Little work, however, has been done on the microflora of the alimentary tract.

Therefore, as one of the basic studies on these problems, the present work was undertaken to investigate methods for the culturing of bacteria present in the intestine of chickens.

Cultural methods used were as follows: Samples for bacteriological examination were collected from the duodenum, middle intestine, cecum, and rectum of chickens sacrificed. A 1 gram portion of the contents of each segment was weighed, and added to 9 ml of sterile saline solution in a test tube. From the initial 10^{-1} dilution thus prepared, serial dilutions up to 10^{-5} were made in sterile saline. The following media was used. (1) Nutritional agar plates. (2) Horse blood agar plates. (3) Glucose

horse blood agar plates. (4) Glucose horse blood liver agar plates. (5) Glucose sheep blood liver agar plates. (6) Tomato juice agar plates. (7) Thioglycollate agar plates. Plates (1) to (4) were incubated both aerobically and anaerobically, and (5) to (7) were incubated only anaerobically. One drop each of suitable diluted samples was spread on these plates with glass rods, and incubated. Anaerobiosis was produced by the method of the phosphorus jar. All the plates were incubated for 4 to 5 days at 40° to 41°C.

Comparative studies on the cultural methods showed the following results:

1) Anaerobic incubation was essential for the growth of intestinal bacteria, since most of them are anaerobic.

2) Among the eleven kinds of plates used, glucose horse blood liver agar plate, incubated anaerobically, was the most excellent for the kinds, numbers, and growth of intestinal bacteria.

3) It was necessary to incubate the plates for 4 to 5 days for differentiation and identification of the bacteria.

Part 2. The bacterial flora of the intestine of healthy chickens. Yuichi OCHI and Tomotari MITSUOKA (Jap. Journ. Vet. Sci. **20**, 45–51, 1958)

To elucidate any interrelationships that may exist between the microorganisms in the alimentary tract and the host, the previous study was made on methods for the culturing of bacteria present in the intestine of chickens as a basic study. The present work was undertaken to determine kinds and numbers of the intestinal flora of healthy chickens at various levels of the intestinal tract.

The experiments were made on 30 chickens during the 3 periods, that is, Apr. to Jun. 1956, July 1956, and Feb. to Mar. 1957. All 30 chickens were Single Comb White Leghorns aged 15 months or more. Both sexes were used. The ration given was Seikei mash No. 1 produced by the Nippon Scientific Compound Feed Company. The methods of the preparation and inoculation of samples were almost the same as in the previous report. Glucose horse blood liver agar plates were incubated anaerobically at 40° to 41°C for 4 to 5 days.

Results obtained from this study were as follows:

1) In general, the intestinal flora of healthy chickens showed similar types in all the different chickens or periods.

2) Lactobacilli (facultative anaerobic lactobacilli) were the most predominant bacteria in the duodenum, comprising almost the entire flora, and few or no other bacteria were found.

3) The flora of the middle intestine were very similar to those of the duodenum except that *Enterobacteriaceae* or *Streptococcus* were found in a slightly greater per cent in some chickens.

4) *Bacillus "D"* obligate anaerobic, Gram positive, non-spore-forming rods including *L. bifidus*) was the most predominant bacteria in the cecum of many chickens, making up about 70 to 100 per cent. of the total cultivatable flora, but few or no *Bacillus* "D" were found in the cecum of some chickens.

5) The flora of the rectum were similar to those of the middle intestine except that *Enterobacteriaceae, Streptococcus, Bacillus "D"*, or others, were increased in per cent. to the total cultivatable flora.

6) The total bacterial counts were found to be the highest in the contents of the cecum and progressively lower in the contents of the rectum, middle intestine and duodenum.

7) Among the apparently healthy chickens, there were a few chickens that had significantly different intestinal flora from those found in other chickens.

156. Studies on Lactobacilli. Yuichi OCHI and Tomotari MITSUOKA (Jap. Journ. Vet. Sci. **20**, 71–86, 1958)

Lactobacilli are widely distributed in nature and commonly found in milk, many dairy and plant products, and the alimentary tract of various animals. The importance of lactobacilli in the field of medicine, veterinary science, and fermentation industries has been accepted for many years. Since the end of the nineteenth century, there have been many studies relating to lactobacilli which were concerned with only the isolation and characterizaton of lactobacilli from particular sources, but the classification of the whole genus has received less attention.

Although, a few systems for the classification have been published recently (Tittsler et al. 1947, Rogosa et al. 1953, Briggs 1953, and Davis 1955), much confusion still exists.

The object of the present work is to establish comprehensive classification and characterization of lactobacilli. Two hundred and twelve strains of the facultative anaerobic lactobacilli and ninty-one strains of *Bacillus "D"* (the obligate anaerobio, Gram-positive, acid producing, non-spore forming rods isolated from the alimentary tract, including *L. bifidus*) were studied. Of these, twenty-six strains were named stock cultures, the others were freshly isolated from various sources.

Results obtained from this study were as follows:

1) *Bacillus "D"* differed sufficiently from the facultative anaerobic lactobacilli in morphology, anaerobic requirement, period of maximum growth, growth on pH 5.0 or 4% NaCl media and survivial duration, to justify clasifying it as a separate genus. The generic name *Lactobacillus* should be reserved only for the facultative anaerobic lactobacilli, while the generic name *Bifidobacterium* (Orla-Jensen, 1924) should be applied to *Bacillus "D"*.

2) Lactobacilli were classified by 3 tests, namely; gas formation from glucose, growth at 15°C, and colony types on glucose horse blood liver agr incubated anaerobically. In case these reactions were not clear, additional tests, namely, the reactions in litmus milk, colony types on glucose sheep blood liver agar incubated aerobically, cell types, growth at 45°C and sugar fermentations, were available.

3) Each type of lactobacilli classified by these criteria was closely correlated with its natural habitat.

4) *Bifidobacterium* was classified into 4 types by means of colony types and cell types. But a complete classification of the genus must be achieved by further studies.

157. Studies on bovine mastitis. Part 1. Normal bacterial flora of the udder of healthy cows. Yuichi OCHI and Yasuji KATSUBE (Jap. Journ. Vet. Sci. **20**, 39–44, 1958)

There are quite a number of reports published on bovine mastitis, but its causes have not been fully explored and, accordingly, its etiology still remains practically unestablished.

As one of basic studies on bovine mastitis, the normal bacterial flora of the udder of healthy cows was first investigated in the present study.

Cows with udders free from any clinical abnormalities, and which produced milk without any macroscopical changes, were regarded healthy. The survey was conducted on the milk samples collected from a total of 301 quarters of 78 healthy cows belonging to 4 herds, bred in the vicinity of Tokyo. Milk samples were inoculated on both nutrient and blood agar plates, each in an amount of 0.02 to 0.03 cc, and were cultured under aerobic conditions at 37°C for 48 hours. Besides the bacterial count, pH determination and cell count were made on each of them.

1. Bacterial isolation from the foremilk was positive in 297 (99%) out of the 301 quarters investigated.

2. The number of viable organisms per cc of foremilk differed considerably depending on the quarter examined.

3. Bacteria belonging to the following families were recovered from the foremilk with a frequency of appearance in the order listed:

1. *Micrococcaceae* 95%
2. *Bacillaceae* 53%
3. *Corynebacteriaceae* 39%
4. *Lactobacillaceae* (*Streptococcus*) 35%
5. Gram-negative bacilli 8%
6. Fungi 1%

Bacteria of similar kinds were also detected in the middle milk and strippings with a slight decrease in number.

4. Mostly, the pH of foremilk was weakly acid except in a few cases (2%) in which it was alkaline.

5. Cell count varied considerably depending on the quarter examined. In most cases, however, the count was below 500,000/cc, but not a few cases, showed a count of over 500,000.

6. It was extremely difficult to determine the normal range of the bacterial count, cell count and pH concentration of normal milk as these figures showed a considerable variation according to the difference in quarters examined.

Part 2. Bacterial flora in the udders of mastitis cows. Yuichi OCHI and Yasuji KATSUBE (Jap. Journ. Vet. Sci. **20**, 83–86, 1958)

As one of the basic studies of bovine mastitis, the authors reported previously on the normal bacterial flora in the udders of healthy cows. In this report, the basterial flora of the cows suffering from mastitis were investigated for clarification of the causative agents of the disease.

Examinations were made on 100 udders of cows found to have suffered from mastitis on post-mortem examination. All the udders showed macroscopic, purulent lesions and in some cases (7) abscesses were formed. Cultures were made from these lesions on both nutrient agar and blood agar plates, for 48 hrs. at 37°C under aerobic contition.

1. Bacterial isolation from the lesions was positive in 97 out of the 100 udders examined.

2. Basteria belonging to the following families were recovered from the lesions with rate of appearance in the order listed.

Lactobacillaceae (*Streptococcus*)	75%
Bacillaceae	51%
Micrococcaceae	43%
Gram-negative bacilli	39%
Corynebacteriaceae	20%

3. *Lactobacillaceae* (*Streptococcus*), *Micrococcaceae*, Gram-negative bacilli and Corynebacteriaceae were isolated from 30, 5, 4 and 3 cows respectively, in the state of almost pure culture, or, in combination with a small number of *Bacillaceae*. Out of the 55 cows from which 2 or more kinds of micro-organisms were isolated, aside from *Bacillaceae*, *Lactobacillaceae* (*Streptococcus*) was predominant in 21, *Corynebacteriaceae* in 8, Gram-negative bacilli in 5 and *Micrococcaceae* in 4.

4. The above mentioned results were summarized from date obtained on two occasions, the first from July to November 1955 and the second from May to November 1957. No considerable difference was observed in the organisms recovered between the time of those two investigations.

Part 3. Classification of *Micrococcaceae* and other organisms isolated from bovine udders. Yuichi OCHI and Yasuji KATSUBE (Jap. Journ. Vet. Sci. **20**, 159–169, 1958)

The authors reported previously on the bacterial flora in the udders of healthy and mastitis cows.

In consideration of the results obtained, it was strongly felt that in order to ascertain the causes of mastitis a more detailed classification should be made of the organisms isolated from both normal and diseased cases.

In this connection, in the present report, *Micrococcaceae* were classified in detail and the results obtained were as follows:

1. Taxonomic studies were made on 212 strains of *Micrococcaceae*.

1) *Micrococcaceae* were classified into 3 large groups, viz., (a) those which decide in two planes at right angels from each other forming tetrads, (b) those which divide in three planes at right angles from each other, forming regular packets, and (c) those which divide in indefinite planes forming irregular masses on the basis of the arrangement of cells.

In the past, the microorganisms forming tetrads were designated as *Gaffkya* and those forming regular packets as *Sarcina*.

As there was no reason to classify irregular mass-forming cocci into Staphyloccus and *Micrococcus*, these cocci were termed *Staphylococcus*.

2) *Staphylococcus* were classified into 3 types on the basis of coagulase production and their behaviour in carbohydrates fermentation.

Strains which produced coagulase and fermented carbohydrates were called *Staphylococcus type I*, those which did not produce coagulase, but fermented carbohydrates, were called *Staphylococcus type II*, and those which did not produce coaglase nor fermented carbohydrates were called *Staphylococcus type III*.

Strains clasified as *Staphylococcus type I*, *Staphylococcus type II*, *Staphylococcus type III*, *Gaffkya* and *Sarcina* were 51, 104, 4, 19 and 34 respectively.

2. Classifications of *Lactobacillaceae* (*Streptococcus*) and *Corynebacteriaceae* were made according to the classification proposed by Ochi et al. (1958, 1940) and the classification of Gram-negative bacilli by the Bergey's Mannual, 7th Ed.

1) Out of the 150 strains of *Lactobacillaceae* (*Streptococcus*) examined, 120 strains were classified as belonging to the *Lactococcus* group, 25 strains to the *Enterococcus* group and the remaining 5 strains to the *Streptococcus* group.

2) All of the 51 strains of *Corynebacteriaceae* were found to belong to *Corynebacterium* and 28 strains were classified as being of *Corynebacterium pseudodiphthericum type I*, 14 as of *Corynebacterium pyogenes* and 9 as of *Corynebacterium pseudodiphthericum type II*.

3) Out of the 33 strains of Gram-negative bacilli available for examination, 17 strains were classified as *Paracolobactrum*, 3 strains as *Escherichia* and 13 as non-identified Gram-negative bacilli.

Part 4. On the etiology of bovine mastitis. Yuichi OCHI and Yasuji KATSUBE (Jap. Journ. Vet. Sci. **20**, 171–177, 1958)

The authors previously reported on the bacterial flora of the udders of healthy and mastitis cows, as well as on the bactetiological classification of the isolated organisms.

I. Comparison of the bacterial flora of the udders of healthy and mastitis cows. As for the normal flora of the udders (3 herds, 53 cows, 201 quarters) *Staphylococcus* was the most predominant and *Bacillus, Corynebacterium, Streptococcus, Gaffkya, Sarcina*, Gram-negative bacilli and Fungi were isolated with a lessening frequency in the order listed. *Staphylococcus, Streptococus, Bacillus, Gaffkya, Corynebacterium* and Gram-negative bacilli were isolated in the state of either pure culture or predominant to the other organisms at the rate of 42, 9, 6, 5, 4 and 1%, respectively. Two or more kinds of organisms were isolated in an almost equal quantity in 29%, and no viable organisms were isolated in 2%.

As for the bacterial flora of mastits cows, *Streptococcus* was isolated most frequently and *Bicillus*, Gram-negative bacilli, *Staphylococcus, Corynebacterium, Sarcina* and *Gaffkya* were isolated less frequently in the order listed. *Streptococcus, Corynebacterium, Staphylococcus* and Gram-negative bacilli were isolated in the state of either pure culture or predominant to the other organisms in 51, 11, 9 and 9%, respectively. Two or more kinds of organisms were isolated in an almost equal amount in 17% and bacterial isolation was negative in 3%.

II. Relation between our classification of the organisms isolated from normal udders and bovine mastits.

1) *Streptococcus*

Lactococcus group was predomniant in both the healthy or the diseased udders, next in frequency was *Enterococcus* group and the least frequent was *Streptococcus* group.

2) *Staphylococcus*

As for the normal flora of udders, *Staphylococcus type II* was observed most frequently (89%), *Staphylococcus type I* came next (9%), *Staphylococcus type III* was least in frequency (1%).

In the case of mastitis, *Staphylococcus type I* comprised 40% of the organisms

isolated and *Staphylococcus type II*, 60%. Out of the 9 cases from which hte *Staphylococcus* was isolated in the state of either pure culture or predominant to the other organisms, 8 cases were *Staphylococcus type I*, while only 1 case was *Staphylococcus type II* and this moreover, was isolated with a considerable amount of *Escherichiae*.

3) *Corynebacterium*

All the organisms isolated from normal udders were *Corynebacterium pseudodiphthericum*, whlie the organisms isolated from cases of mastitis, 70% were identified as *Corynebacterium pyogenes* and 30% as *Corynebacterium pseudodiphthericum*.

In the case of mastitis, out of the 11 cases from which *Corynebacterium* was isolated in the state of either pure culture or predominant to other organisms, 9 cases were *Corynebacterium pyogenes* and 2 cases were *Corynebacterium pseudodiphthericum*, one being mixed with *Streptococcus* and the other with *Escherichiae* and *Streptococcus*.

4) Gram-negative bacilli

A few strains isolated from normal udders were identified as *Enterobacteriaceae*, while many strains isolated from the cases of mastitis were identified as *Esherichiae*.

In the case of mastitis, out of the 9 cases from which Gram-negative bacilli were isolated in the state of either pure culture or predominant to the other organisms, 8 cases were *Echerichiae* and 1 case was *Proteus*, mixed with *Streptococcus*.

Isolation of *Gaffkya* and *Sarcina* was low in frequency and *Bacillus* was rather high from both normal and mastitis udders. In the case of mastitis, however, these organisms were not isolated in the predominant state.

On the basis of the above mentioned results, *Streptococcus*, especially *Lactococcus* group, *Staphylococcus type I*, *Corynebacterium pyogenes* and *Esherichiae* are regarded as the most important causative agents of bovine mastitis.

Since all the causative agents of bovine mastitis are the organisms found in the normal flora, the authors consider that bovine mastitis is a disease caused not merely by the organisms themselves but requires some predisposing factors placed in the udders which allow active multiplication of these organisms and suggest that it is a polybacterial non-specific disease which can be included in Ochi's theory of autogenous infectious diseases.

158. Studies on therapeutic prophylaxis for immature worm at the latent period of *Schistosomiasis japonica*. Shin Okoshi, Mampei Usui, Isamu Tomoda, Noritoshi Kitano and Mitsuo Takashio (Jap. Journ. Parasitol. 7, 3, 1958)

Dogs were infected artificially with cercariae of *Schistosoma japonicum* and treated in the latent period, during which, passage of the eggs in the faces was not observed, with injection of sodium antimony tartrate, as a prophylactic means to kill the immature worms of the parasite and to prevent the onset of schistosomiasis japonica.

The animals were infected artificially with cercariae of the parasite, at the rate of about 100 organisms per kilogram of body weight respectively and divided into four groups; the first group which was treated instantly after the infection, the second group which was treated since 10 days after the infection, the third group which was treated since 20 days after the infection, and the fourth group which was

treated since the day when passage of the eggs in the feces was confirmed.

Doses of sodium antimony tartarate, including 3, 6, 9 and 12 mg. of antimony per kilogram of body weight in total, were injected intravenously several times to the animals of each group. Fecal examinations of the animals were made by the M.I.F.C. method everyday since the day of the first injection. At the end of the experiments, all the animals were killed for pathologo-anatomical examination and examined scrupulously to confirm presence or absence of the worms and eggs.

1) In the first group, which was treated instantly after the infection, all animals passed eggs and the injection was inffective, irrespective of the quantities of total doses injected. However, the day of the first passage of egg in the feces was delayed in the case animals treated with more doses of the drug than in the ones with less doses.

2) In the second group, which was treated with the drug since 10 days after the infection, in animals treated with total doses of 3-6 mg./Kg. of antimony the egg passage in the feces was observed, but in animals treated with total doses of 9-12 mg./Kg. the worms were successfully killed and the onset of the disease was prevented.

3) In the third group, which was treated since 20 days after the infection, in animals treated with total dose of 3 mg./Kg. the egg passage in the feces was observed, but in animals treated with total doses of 6-9 mg./Kg. or more, the worms were successfully killed and the onset of the disease was prevented.

4) In the fourth group, (which was treated since 33 days after the infection or later, or the day when the passage of eggs in the feces was first confirmed) where the animals were treated with a total dose of 10 mg./Kg. or less, the egg passage in the feces became negative for some time, but was again observed 50-88 days after the infection. In animals treated with total doses of 12-15 mg./Kg. the adult worms were all killed completely and the egg passage in the feces was stopped.

From the above mentioned it might be concluded; in animals of the group (Ist) which was treated instantly after the infection, injection of sodium antimony tartarate including 12 mg./Kg. or less of antimony in total did not kill the worms, but in animals of the group (IIIrd) which was treated since 20 days after the infection, injection of the drug including 6-9 mg./Kg. of antimony was able to kill the immature worms durig the latent period.

159. Studies on the electrophoretic patterns of *Schistosoma japonicum* infecting rabbit and canine sera, especially on the changes in the treatment of antimony compound and reinfection. Shin OKOSHI, Isamu TOMODA, Mampei USUI, Noritoshi KITANO and Mitsuo TAKASHIO (Jap. Journ. Parasit. 7, 279-280, 1958)

Sera from rabbits and dogs, experimentally infected with Schistosoma japonicum were fractionated in the filter paper electrophoresis system, in an attempt to ascertain the changes of the serum protein component concentrations in the treated animals.

Dogs and rabbits used in the experiment were all normal, adult and male, and weighed 10-15 Kg. and 2-3 Kg. respectively. The artificial infections were made as follows: the infective cercariae of approximately 200-300 per kilogram of body weight from several crushed snails were placed on the shaved area of the abdomen of the animals.

Dose of the antimony compound, sodium antimony tartrate, including a daily 0.5 mg per kilogram of body weight of antimony in the dogs, were administrated intravenously to 6 schistosome infected dogs and 3 uninfected dogs for 24 days from the 45th day of infection. While, in the rabbits, dose of the antimony compound, including daily 1 mg per kilogram of body weight of antimony, were administrated intravenously to 5 schistosome infected rabbits and 6 uninfected rabbits for 20 days from the 50th day of infection.

Experimental reinfection was made in the treated animals 100 days after treatment by a similar method as in the initial infection.

Blood was collected at 10 day intervals for 170 days following infection in the initial infection, and for 60 days following infection in the reinfection. As controls, the samples from the nontreated schistosome infected group (14 dogs and 15 rabbits) and nontreated uninfected group were collected at the same intervals during the experiment. Sera was recovered by centrifugation, and total protein determinations were made by the Hitachi reflactometer and serum proteins were fractionated by the horizontal bridge electrophoresis system.

Results were as follows:

No notable changes in the serum protein fractions were observed in the early stage of infection, i.e., for 40 days following infection. However, on the 50th day after infection, a sharp differentation between the control and experimental ionograms, i.e., the marked increase in total protein, beta- gamma-globulin and relative decrease in albumin, was observed. After this, the above serum protein responses were observed more significantly with progressive schistosoma infections in the nontreated groups.

While, in the antimony treated groups, the above serum protein responses, i.e., the increase in total protein, beta- gamma-globulin and the decrease in albumin, were observed still in general, similar to the nontreated animals during the treatment, however, following 20-30 days after treatment, the tendency of the decrease in total protein, and beta- gamma-globulin and the relative increase in albumin was observed, and the serum protein component concentrations of the treated animals were approached or was at the control level on the 100th day after treatment.

Above changes in the serum protein fractions were observed markedly in the rabbts especially, anr in the heavy cases, the recovery of altered serum protein patterns was not significantly observed. Through the course of the experiment, there was relative decrease in albumin, but the absolute concentration of this component remained approximately within the biological limits. Therefore, with the progressive disease, the increase in total protein was found referable by ionographic determination directly to the absolute increase in the beta- gamma-globulin regions.

In the reinfected rabbit and canine sera, the increase in total protein, beta- gamma-globulin and the decrease in albumin were observed in general similar to the initial infected animal sera from 50 days after infecton.

Accordng to the above results, when administrated to the normal dogs and rabbits at similar doses of the antimony compound, the significant changes of the serum protein component concentrations were not observed in general, though the slight changes were obtained during the treatment and at the post-treatment.

Also, as the liver function tests, B.S.P., Takata, Cadmium, Cobalt and Gros reactions showed positive in all infected cases, a marked increase of gamma-globulin

was observed which could be attributed to the liver damages. And then, these reactions showed more marked positive, according to the progress of the disease.

160. Studies on the serum protein patterns in cattle infected wirh *Schistosoma japonicum*, especially about the effect of antimony compound on serum protein. Shin OKOSHI, Isamu TOMODA, Mampei USUI, Mitsuo TAKASHIO and Noritoshi KITANO (Rep. 17th Meeting East Div., Jap. Soc. Parasit. 7, 1957)

Sera from cattle infected with Schistosoma japonicum were analyzed by filter paper electrophoresis 3 times; (1) prior to administration, (2) just after, and (3) 32 days after treatment following administration of sodium antimony tartrate, in an attempt to find an explanation for the variations of the serum proteins in animals subjected to antimony compound action during the course of Schistosoma japonicum infection.

Dose of sodium antimony tartrate, including 3 mg. of antimony per kilogram of body weight in total, was injected intravenously. Total protein determinations were made by the reflactometer and serum proteins were fractionated by the filter paper electrophoresis system.

Results were as follows.

1) The average values of serum protein component concentrations obtained from 13 cases of Japanese native cattle infected with Schistosoma japonicum prior to administration of the antimony compound were: total protein 6.6±0.49 g/dl; albumin 39.5±3.78% (2.6±0.24 g/dl); alphaglobulin 14.8±0.87% (1.0±0.09 g/dl); betaglobulin 16.1±1.11% (1.0±0.15 g/dl); gammaglobulin 29.6±3.50% (2.0±0.38 g/dl); and A/G ratio 0.67±0.11. Therefore, the increase of gamma-globulin, the decrease of albumin and the resultant marked lowering of the A/G ratio in the schistosome infected cattle sera was observed as compared with the same fractions in the uninfected cattle sera.

Gros, Cadmium and Cobalt reactions for the liver function test showed the marked positive in the infected cattle sera.

2) The tendency of decrease in gamma-globulin and relative increase in albumin was observed in the protein fractions of treated cattle as compared with the non-treated cattle through the course of experiment.

In the examination of parasite ova by the rectal mucous membrane scratching method about the 11 cattle on the 32nd day after treatment, four cases were negative for parasite ova, and the other were still positive.

The average values of the serum protein component concentrations obtained from 11 cattle on the 32nd day after treatment were as follows: total protein 6.5±0.56 g/dl; albumin 41.6±3.97% (2.6±0.17 g/dl); alphaglobulin 15.6±1.53% (1.0±0.10 g/dl); betaglobulin 16.2±1.79% (1.0±0.21 g/dl); gammaglobulin 26.5±3.46% (1.7±0.38 g/dl); A/G ratio 0.72±0.10. And, on the 32nd day after treatment, the serum protein patterns of them still showed the hypergammaglobulinemia and hypoalbuminemia.

3) In addition to the above, the samples of nine cases of normal Japanese native cattle were submitted to the filter paper electrophoretic analysis and the following results weer obtained: total protein 6.1±0.22 g/dl; albumin 51.2±2.63% (3.1±0.15 g/dl); alphaglobulin 15.1±1.54% (0.9±0.07 g/dl); betaglobulin 13.8±1.54% (0.8±0.10 g/dl); gammaglobulin 19.9±2.61% (1.2±0.33 g/dl); A/G ratio 1.06±0.05.

161. Studies on the effect of ammonium germanate on the blood pictures in dogs. Shin OKOSHI, Mampei USUI, Masanobu FUKUI and Takeo TAKAHASHI (Journ. Jap. Vet. Med. Ass. **10**, 473, 1957)

Fifteen dogs were administered intramuscularly and intravenously with water solution of ammonium germanate at doses of 0.3-0.5 mg./Kg. of germanium.

In every case, the total leucocyte counts showed increases of 23-64% during the period of 4 to 12 hours after the injection. This increase was mainly due to the increase of neutrophils. The absolute counts of eosinophils, lymphocytes and monocytes showed little changes. The erythrocyte counts did not show remarkable changes.

162. Studies on the diffusion of sulfamin into the vaginal fluid and it's concentration in the blood in dogs following administrations of various sulfamin preparations. Shin OKOSHI, Isamu TOMODA, Toshio ISHII and Mampei USUI (Journ. Jap. Vet. Med. Ass. **10**, 473, 1957)

The purpose of this study was to determine the diffusion of sulfamine into the vaginal fluid comparing with it's blood concentration in the dogs following intravenous or oral administration of five sulfamins—Sulfathiazole, Sulfamerazine, Sulfadiazine, Thiazin and Domian.

The sulfonamides were administered at the rate of 100 mg per kilogramm or 200 mg per kilogram of body weight intravenously as a single dose. Samples were taken at regular intervals for 24 hours after administration. The total sulfamin contents of these samples were determined according to the method of Bratton-Marshal.

When given orally, the sulfamin concentrations in the vaginal fluid were lower than the blood levels, and ranging from 1/2 to 1/3 of the latter through the course of experimental period.

Thiazin and Domian, when administrated intravenously, were rapidly defined in the vaginal fluid, and increased to the maximum vaginal fluid concentrations one or two hours after administration, and then decreased rapidly after a few hours according to the blood concentration declining. While, the blood concentrations maintained the therapeutic levels for about four to six hours.

Following continued administration, the blood and vaginal fluid concentrations could maintain the approximate constant levels for a long time.

163. The effect of the artificial daylight (ultra-violet ray) on the blood pictures and urine components in dogs. Shin OKOSHI, Mampei USUI, Masanobu FUKUI, Isamu TOMODA and Hiroshi ITAGAKI (Journ. Jap. Vet. Med. Ass. **10**, 445, 1957)

We observed the changes of blood pictures and urine components of nine dogs following the irradiation with ultraviolet ray produced by mercury vapour lamp. Radiation time was 30 minutes and distance was 30 cm. or 50 cm.

The total leucocyte counts showed an increase of average 30% during the period of 2 to 6 hours after the irradiation. This increase was mainly due to the increase of neutrophils. Blood platelet counts also showed an increase of the same condition with total leucocyte counts. The erythrocyte counts and the hemoglobin contents did not show remarkable changes, but in three dogs a slight incrase of the erythrocyte counts was observed after 1 week from the irradiation.

Differences between the reactions by radiation at the distance of 30 cm. and those

at the distance of 50 cm. were not observed.

Three dogs were irradiated two times with an interval of 6 hours. In these cases, reactions by the second radiation were of the same condition as that of the first.

Changes in pH., protein, sugar and aceton body of the urine were not observed in every case.

164. Clinical reviews of toxoplasmosis in dogs and cat. Shin OKOSHI, Takeo TAKAHASHI, Toshio ISHII, Mampei USUI, Isamu TOMODA, Masanobu FUKUI and Hiroshi ITAGAKI (Journ. Jap. Vet. Med. Ass. **10**, 472-473, 1957)

One case of toxoplasmosis in cat and two cases in dogs were observed in the Veterinary Clinic of the University of Tokyo.

The case of a cat, aged 2 years, which showed enlargement of abdomen and anorexia since August 27th, 1954, was accepted, in the clinic on September 7th of the same year. Much quantity of ascites, 150 per minute of pulse rate, 38.7°C of body temperature, 120 per minute of respiration rate, none of parasitical ova, normal feces and normal urine, were the symptoms then observed. The animal was treated with exclusion cf 200 cc. of ascites, and with injections of penicilline, vitamins, glucose and cardiac, however, it died on September 11th of the same year. Toxoplasma organisms were discovered in the ascites.

One of the two cases of dogs, a male shepherd, 3 months old, and the other a female of the same breed, 2 months old, were both raised by the same owner. The male was affected since November 13th, 1954 and died on December 6th of the same year, while the female was affected since November 17th and euthanised on December 7th of the same year.

High fever and the symptoms of distemper complex were observed, and hyperkeratosis of pads and the 'tick' spasms were developed since a week prior to death. All kinds of antibiotics like penicilline, mycin, or chemotherapeutics such as sulfamine were all used ineffectively. Ascites were not developed in both cases. Toxoplasma organisms were discovered in their lungs, lymph nodes of plumonary hilum, spleens, pancreases and bone marrow.

165. Studies on the incidence of Fasciola hepatica and Paramphystomum spp. in Japanese native oxen (Mishima breed). Shin OKOSHI, Toshio ISHII, Isamu TOMODA, Noritoshi KITANO, Ken YAMAMOTO, Katsumi HIGACHI and Hiroshi NAKAMURA (Journ. Jap. Vet. Med. Ass. **10**, 471, 1957)

Mishima island is 25 miles off the Sea of Japan from Hondo (Japan proper). As oxen have not been imported to this island for long times, the Mishima oxen are fit for the studies of Japanese native oxen.

The examination on Fasciola hepatica and Paramphystomum spp. in Mishima oxen were performed according to the fecal test by Watanabe's method, Ono's intradermal test and our method by allergen made from adults of the above trematodes. 233 heads of oxen, 187 from Honmura and 46 from Utumura, two villages of Mishima is., were tested. The ages of the oxen ranged from 2 to 19.

The fecal test by Watanabe's method showed infestation percentages, 31.3% of F. hepatica, 62.7% P. spp. and 25.3% of mixed infection. No significant differences were observed in the infestation percentages among animals with different age and

of different habitating places. Infestation percentages of F. hepatica by Ono's method were 80.1-57.6% with average of 68.9%, and the goodness of fit between infestation percentages by the latter method and by the fecal test was expressed: Pr=0.25.

166. Studies on the effect of nembutal (pentobarbital) an esthesia in dogs. Shin OKOSHI,* Takeo TAKAHASHI, Kozi TO and Masanobu FUKUI (Journ. Jap. Vet. Med. Ass. **10,** 472, 1957)

Effect of nembutal, an anesthetic made by Abbott, U.S.A. 6% pentobaroital sodium) on dog was as follows;

1) Injection of nembutal of a dosage 0.44 ml. per kg. of body weight intravenously, induced an optimal deep anesthesia for about 1 hour in the animal.

2) The intravenous injection had no side effects, irrespective of the velocity with which it was injected.

3) Body temperature and respiration rate of the animal were decreased during anesthesia.

4) In waking period, forward struggling, excitement and frenzy were observed for several hours.

5) In intravenous injecton of the drug at the doses of 0.45 ml./Kg. or more, th animal might be fatal.

6) In subcutaneous injection of the drug at the dose of 0.44 ml./Kg., deep anesthesia was not attained.

7) In intraperitoneal injection at the dose of 0.44 ml./Kg., the animal was in a state of hypoesthesia, just between states caused by intravenous injection and subcutaneous injection.